Cranfield University

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Disinfection of grey water

School of Applied Sciences

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ABSTRACT

The reuse of grey water, for applications such as toilet flushing and irrigation, represents a potential sustainable solution to water shortages experienced by regions worldwide. Although reused grey water is not intended for potable use, the potential for transmission of waterborne pathogens by aerosol inhalation, topical contact, or indirect ingestion is a key concern for grey water reuse. This thesis explores the pathogen content of grey water and investigates pathogen removal through treatment and disinfection processes. The impacts of organic and particulate material in grey water on the efficacy of disinfection processes are investigated in depth.

Grey water can potentially harbour a range of pathogenic microorganisms, with opportunistic bacterial pathogens in grey water indicating a particular risk of grey water reuse for the vulnerable members of society. The disinfection of grey water is therefore critical prior to reuse. Particulate material in grey water limits the efficacy of disinfection by chlorine, ultraviolet light, and origanum essential oil, by shielding microorganisms from the applied disinfectant. Microbial resistance to the disinfectants was linked to the particle size distribution of the grey water, with increasing particle size offering greater protection to associated microorganisms. Additional organic material was shown to reduce the applied disinfectant but no impact on microorganism resistance to disinfection was observed when a constant disinfectant dose was maintained. Treatment of grey water, targeting the removal of large particulate material, improves the efficacy of grey water disinfection, allowing compliance with stringent microbiological standards for urban water reuse.

ACKNOWLEDGEMENTS

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Thanks to everyone at the Centre for Water Sciences who helped with my work in some way, or helped to take my mind off it! For that, Dan deserves a mention for conscientiously ensuring that I attended morning coffee break, or "toast time", every day, without fail. Special thanks go to the occupants of PhD Room 3, notably Charlie, Ewan, Nafsika, and Rita, who not only humoured my numerous stupid questions but who also made life at Cranfield that much more enjoyable.

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ABBREVIATIONS AND NOTATION

Abbreviations

BOD – Biological oxygen demand

COD – Chemical oxygen demand

EO – Essential oil

GROW – Green roof water recycling system

HFRB – Horizontal flow reed bed

HLR – Hydraulic Loading Rate

HRT – Hydraulic Retention Time

MBR – Membrane bioreactor

MCR – Membrane chemical reactor

RPM – Revolutions per minute

TOC – Total organic carbon

TSS – Total suspended solids

UK – United Kingdom

UV – Ultraviolet light

VFRB – Vertical flow reed bed

Notation

D[4,3] – volume weighted mean particle size

D10, D50, and D90 – particle diameter below which lies a percentage (10, 50, or 90 %) of the total volume of all particles in the sample

dN_t/dt – the rate of change in the number of coliforms with time

E – Energy

h – Planck's constant

k – inactivation rate constant

N_t – the number of coliforms at time t

t – time.

v – frequency

CHAPTER 1:
INTRODUCTION

1. INTRODUCTION

1.1. BACKGROUND

An increasing global population coupled with growing urbanisation in many already water-scarce regions worldwide has led to increased demands on water supply (USEPA, 2004). In the southern United Kingdom, for instance, economic growth and extensive house building is putting water supply under increasing pressure, with the efficient use of water deemed "crucial to successful water resource management over the next 25 years" (Environment Agency, 2001). The recycling and reuse of water is therefore imperative in some areas, and increasingly so in others, to meet demand for urban, industrial and agricultural water requirements. The practice of water reuse involves reclaiming water sources that would otherwise be released into the environment and using them for a defined purpose. Municipal wastewater is often reused following advanced treatment at large-scale specialised wastewater reclamation plants. For instance, in Japan, around ten percent of wastewater treatment plants provide effluent for reuse, and some $8.5\times10^{7}m^{3}$ effluent is reused each year, following advanced treatment (Maeda *et al.*, 1996). An alternative strategy for urban water reuse is decentralised water management, whereby the collection and treatment of water occurs on a smaller scale and close to the point of reuse. Decentralised urban water reuse schemes range from individual homes or clusters of homes, to institutional or commercial facilities (Tchobanoglous and Angelakis, 1996). Common applications for reused water include: toilet flushing, irrigation and vehicle washing (USEPA, 2004).

Grey water, which can be defined as all in-building wastewater streams, with the exception of toilet wastewater, is a potential water source for decentralised urban reuse. Alternative water sources include black water (grey water + toilet wastewater) and rain water, however, the reuse of grey water holds a number of advantages. First, it is continually and consistently produced onsite, meaning a readily available and reliable source of water is available for reuse. Secondly, grey water is produced in sufficient quantities for reuse applications. For example, 69% of in-building wastewater is

discharged as grey water and 31% as toilet wastewater (Almeida *et al.*, 1999), indicating that reused grey water can provide sufficient water for household toilet flushing in addition to other applications, such as garden irrigation. Thirdly, by excluding toilet wastewater, grey water is less polluted (Almeida *et al.*, 1999); eliminating the potential problems associated with toilet paper waste, and likely improving its image in terms of public perception. A principal concern for water reuse is the potential for the transmission of pathogenic microorganisms from reuse applications (Asano, 1998). Pathogens of concern include those commonly associated with disease outbreaks from recreational waters, such as *Cryptosporidium*, *Pseudomonas* spp. and *Shigella* spp. (Craun *et al.*, 2005). Knowledge of the pathogen content of grey water is limited. However, specific pathogens and significant numbers of indicator bacteria have been reported (Rose *et al.*, 1991; Birks *et al.*, 2004; Jefferson *et al.*, 2004), indicating that the disinfection of grey water prior to reuse is essential to reduce the risk to public health.

The present thesis reports work contributing towards the WaND (Water Cycle Management for New Developments) project, funded by the EPSRC (Engineering and Physical Sciences Research Council).

1.2. AIM AND OBJECTIVES

This thesis aims to understand the pathogen content of grey water and pathogen removal from grey water by treatment and disinfection processes to provide safe, pathogen-free water for urban reuse. Accordingly, the following objectives were identified:

1. To assess current knowledge of the pathogens present in grey water and the suitability of disinfection technologies for their removal.
2. To determine the microbial quality and presence of specific pathogens in real grey water
3. To evaluate the microorganism removal performance of leading contender grey water treatment technologies
4. To assess the efficacy of established and novel disinfectants for the disinfection of grey water

5. To investigate the impact of grey water characteristics on the efficacy of disinfection with a view to informing treatment requirements prior to disinfection and reuse

1.3. THESIS PLAN

This thesis is presented in a paper format. All papers were written by the first author, Gideon P. Winward, and edited by Dr. Bruce Jefferson. All experimental work was undertaken by Gideon P. Winward, with the exception of Chapter 3 where some chemical and physical water quality analysis was conducted by Ronnie Frazer-Williams and Marc Pidou.

The thesis begins with a review of the literature on pathogens present in urban waters, principally grey water, and the impact of water quality on the selection of disinfection technologies for urban reuse (Chapter 2 – intended for submission to Urban Water Journal: *Winward, G.P., Avery, L.M., Stephenson, T. and Jefferson, B., Pathogens in urban waters and disinfection options for reuse*).

Chapters 3 to 6 cover the technical content of the thesis. Chapter 3 investigates the microbial quality of grey water, in terms of specific pathogens and indicator bacteria, and the pathogen removal performance of potential treatment technologies for grey water reuse (Chapter 3 – submitted to Ecological Engineering: *Winward, G.P., Avery, L.M., Frazer-Williams, R., Pidou, M., Jeffrey, P., Stephenson, T. and Jefferson, B., A study of the microbial quality of grey water and an evaluation of treatment technologies for reuse).*

Chapter 4 studies the impact of grey water quality, specifically particle size and organic concentration, on the efficacy of chlorine disinfection and discusses the implications for urban water reuse (Chapter 4 – in press in Water Research: *Winward, G.P., Avery, L.M., Stephenson, T. and Jefferson, B., Chlorine disinfection of grey water for reuse: effect of organics and particles).*

Chapter 5 explores the impact of grey water quality on disinfection by ultraviolet (UV) light, further illuminating the relationship between particle size and shielding of microorganisms from the applied disinfectant (Chapter 5 – submitted to Environmental Science and Technology: *Winward, G.P., Avery, L.M., Stephenson, T. and Jefferson, B., Ultraviolet (UV) disinfection of grey water: Particle size effects).*

Chapter 6 assesses the suitability of novel disinfectants for water disinfection: plant essential oils. A range of different essential oils and their key components are screened for disinfection properties and the impact of water quality on the efficacy of disinfection with origanum essential oil is further explored. The use of essential oils for the inhibition of regrowth in treated grey water effluent is also investigated (Chapter 6 – submitted to Water Research: *Winward, G.P., Avery, L.M., Stephenson, T. and Jefferson, B., Essential oils for the disinfection of grey water).*

The thesis concludes in Chapters 7 and 8 with a comparative discussion of the disinfection processes investigated and their application for grey water reuse, before drawing final conclusions and suggestions for further work.

Table 1.1. *Summary of thesis plan.*

Chapter	Title	Journal	Status	Objectives addressed
2	Literature review - Pathogens in urban waters and disinfection options for reuse	Urban Water Journal	In preparation	1
3	A study of the microbial quality of grey water and an evaluation of treatment technologies for reuse	Ecological Engineering	In press	2, 3
4	Chlorine disinfection of grey water for reuse: effect of organics and particles	Water Research	In press	4, 5
5	Ultraviolet (UV) disinfection of grey water: Particle size effects	Environmental Science and Technology	In press	4, 5
6	Essential oils for the disinfection of grey water	Water Research	In press	4, 5
7	Overall issues for reuse	-	-	1, 2, 3, 4, 5

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Tchobanoglous, G. and Angelakis, A.N. (1996) Technologies for wastewater treatment appropriate for reuse: potential for applications in Greece. Water Science and Technology 33(10-11), 15-24.

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CHAPTER 2:
LITERATURE REVIEW – PATHOGENS IN URBAN WATERS AND DISINFECTION OPTIONS FOR REUSE

INTENDED FOR SUBMISSION TO: *Urban Water Journal*

2. LITERATURE REVIEW - PATHOGENS IN URBAN WATERS AND DISINFECTION OPTIONS FOR REUSE

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ABSTRACT

Urban waters, including black water, grey water, and rain water, are all potential sources of water, which can be reused in an effort to conserve limited water resources. Urban waters can contain a range of pathogenic microorganisms, however, which are of concern for water reuse due to the potential for pathogen transmission from reuse applications. Adequate disinfection of urban waters is therefore vital to address the potential health risks of reusing urban waters. A variety of possible disinfection options exist for urban waters with varying efficacies for pathogen elimination. The majority of disinfection processes are also strongly influenced by the water quality, including the quantity of organic and particulate material. The presence, frequency, and concentration of pathogens in urban waters are reviewed, as are the prospective disinfection technologies for urban water reuse. The implications of pathogen content and urban water quality on the selection of disinfection technologies for reuse are discussed.

2.1. INTRODUCTION

Urban water sources available for reuse include: black water (all in-building wastewater streams, including toilet waste), grey water (all in-building wastewater streams, excluding toilet waste), and rain water. The capture and reuse of urban waters is normally conducted to provide water for non-potable in-building applications, including toilet-flushing and laundry; as well as external applications, such as landscape irrigation, car washing, and crop irrigation (USEPA, 2004). This decentralised reclamation and reuse of water, involving individual and clusters of homes, institutions, and commercial premises', is recognised as a viable and sustainable solution to regional

water shortages (Anderson, 1996; Tchobanoglous and Angelakis, 1996; Fane *et al.*, 2002). However, the reuse of urban waters introduces a potential risk of infection to users from waterborne pathogenic microorganisms. Appropriate disinfection of reclaimed urban water is therefore essential to minimise the risk to public health from water reuse.

A variety of bacterial, protozoan and viral pathogens are transmitted via water, and infection may occur through inhalation, ingestion, or topical contact with reused water. A key route of transmission is thought to be through aerosol generation during the agitation of water, which enables the movement of pathogenic microorganisms (Fannin *et al.*, 1985; Goldmann, 2000), facilitating their transmission by inhalation, and their transport onto surfaces, which may subsequently come into contact with, or be ingested by, an individual (Beggs, 2003). Toilet flushing, for example, has been shown to produce aerosols, containing bacteria and viruses, which can settle on surfaces throughout the bathroom (Gerba *et al.*, 1975). For instance, toilet flushing following a simulated diarrhoea event resulted in seeded indicator bacteria and viruses spread up to 83cm from the toilet seat (Barker & Jones *et al.*, 2005).

Toilet flushing and other aerosol-producing applications, such as car washing or spray irrigation, with reused water may therefore carry an additional risk of infection, particularly by opportunistic pathogens that are infectious by inhalation or topical contact, including *Pseudomonas aeruginosa* (Evans *et al.*, 1996), *Staphylococcus aureus* (Wertheim *et al.*, 2005), *Legionella pneumophilia* (Atlas, 1999), and nontuberculoid *Mycobacteria* (Falkinham, 2003). These opportunistic pathogens can cause a range of diseases, primarily respiratory tract infections and skin infections. The elderly, very young, and immunocompromised are the members of society most at risk from these waterborne pathogens and most likely to develop severe disease and complications. *P. aeruginosa* and *Staph. aureus*, for example, are of minor concern to the immunocompetent but can cause severe disease in the vulnerable, evidenced by their prevalence in hospital-acquired infections (Hardalo and Edberg, 1997; Wertheim *et al.*, 2005).

Exposure to pathogens in reused water may also occur as a result of unintended applications for reused water, such as use to fill up paddling or swimming pools, or to water garden vegetables, which are subsequently consumed. The ingestion of water contaminated with enteric pathogens typically results in gastrointestinal illness. Enteric pathogens are excreted in the faeces of infected individuals and transmission occurs via faecally-contaminated water sources. Common enteric pathogens include the bacteria: *Salmonella* spp., *Shigella* spp., *Campylobacter* spp., *Escherichia coli*; the protozoa: *Cryptosporidium* spp., *Giardia* spp., *Entamoeba* spp.; and the viruses: norovirus, saporovirus, rotavirus.

This review investigates the presence, frequency, and concentration of pathogens in urban waters and assesses the implications of urban water quality on the selection of disinfection technologies for reuse.

2.2. PATHOGENS IN URBAN WATERS

2.2.1. Black water

Black water includes all in-building wastewater streams, including toilet waste, and is therefore highly polluted. Average COD (chemical oxygen demand) and TSS (total suspended solids) values for black water are 1094 and 548 $mg.L^{-1}$, respectively (Almeida *et al.*, 1999). Data on indicator bacteria and specific pathogens in directly-sampled black water are sparse but microorganisms reported in untreated wastewater can provide a good indication of the potential microbial quality of black water. Untreated wastewater is typically diluted with urban runoff water, however, and so black water can be expected to be more polluted, in terms of pathogenic microorganisms as well as water quality parameters (Table 2.1.).

Literature data on untreated wastewater indicates that black water contains consistently high concentrations of indicator bacteria as well as specific enteric and opportunistic pathogens. For instance, indicator bacteria concentrations of 7.6 and 6.3 $\log_{10}.100mL^{-1}$

for total coliforms and Enterococci, respectively, are typical in untreated wastewater (Kay *et al.*, 2007, Figure 2.1.). Pathogenic protozoa and viruses, including *Cryptosporidium*, *Giardia*, and enteroviruses, have been reported in untreated wastewater entering treatment plants at average concentrations of 2.6-3.2, 3.6-5.1, and 2.6-6.0 $\log_{10}.100L^{-1}$, respectively (Rose *et al.*, 1996; Rose *et al.*, 2001; Ottoson *et al.*, 2006). *Salmonella* spp. have also been reported, at a mean concentration of 2.4 $\log_{10}.100mL^{-1}$ (Howard *et al.*, 2004). Enteric pathogens are shed in large numbers in the faeces of infected individuals, up to 10^{11} per gram (Leclerc *et al.*, 2004), and thus the presence of significant numbers in black water is expected. Opportunistic pathogens, such as *P. aeruginosa* and *Aeromonas* spp., would also be expected in black water, having been detected in untreated wastewater (Shannon *et al.*, 2007). *P. aeruginosa* was present at a concentration of 5.6 $\log_{10}.100mL^{-1}$ in raw wastewater investigated by Howard *et al.* (2004).

Table 2.1. *Water quality of urban waters.*

Water quality parameter	Grey water (light)	Grey water (dark)	Rain water	Black water	Untreated wastewater	Treated wastewater
BOD ($mg.L^{-1}$)	59 - 424	48 - 890	1.4 – 3.7	-	110 - 350	10 – 30
COD ($mg.L^{-1}$)	100 - 645	361 - 1815	-	1094	250 – 800	-
TOC ($mg.L^{-1}$)	40 - 120	84 – 582	-	-	-	-
TSS ($mg.L^{-1}$)	30 - 303	35 - 625	1 - 153	548	120 – 400	<1 – 30
Ammonia ($mg.L^{-1}$)	<0.1 – 15.0	<0.1 - 4.6	0.01 – 0.1	23	12 - 25	-
Turbidity (NTU)	23 - 240	103 - 148	0.6 – 56	-	-	1 – 30
pH	6.4 – 8.1	5.2 – 10.0	6.4 – 8.3	-	-	-

References:
Grey water: Christova-Boal et al. (1996); Fittschen and Niemczynowicz (1997); Surendran and Wheatley (1998); Almeida et al. (1999); Nolde (1999); Casanova et al. (2001b); Jefferson et al. (2004); Ramon et al. (2004); Dallas and Ho (2005); Friedler (2004); Friedler et al. (2006); Grant et al. (2006); Merz et al. (2007).
Rain water: Yaziz et al. (1989); Simmons et al. (2001); Adeniyi and Olabanji (2005); May and Prado (2006); Sazakli et al. (2007).
Black water: Almeida et al. (1999).
Municipal wastewater: Tchobanoglous et al. (2003)
Treated wastewater: Asano (1998).

Treated wastewater is also a source of water for reuse and data on treated effluents is included here to serve as a benchmark for comparison with urban waters. Despite significant reductions in water quality parameters (Table 2.1.), treated wastewater effluents can retain high levels of indicator bacteria. For example, total coliform

concentrations in secondary treated wastewater effluents range from 2.6 to 7.6 $log_{10}.100mL^{-1}$ and the faecal indicators, *E. coli* and Enterococci, from 1.7 to 5.7 $log_{10}.100mL^{-1}$ (Figure 2.1.). Further information regarding the microbial quality of wastewaters can be found elsewhere (Asano, 1998; Kay *et al.*, 2007).

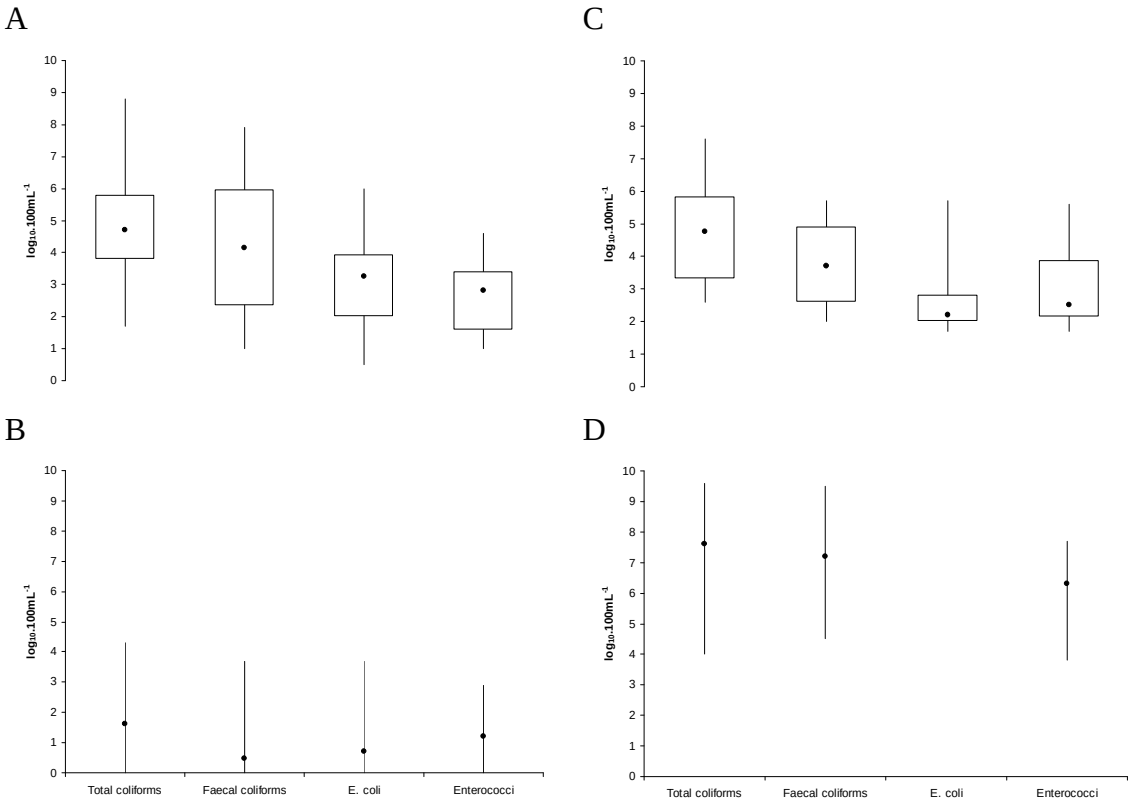

Figure 2.1. *Indicator bacteria in grey water (A), rain water (B), treated wastewater (C), and untreated wastewater (D). Box and whisker plot represents the median* (●), 25th and 75th percentiles (lower and upper edges of box), and the minimum and maximum values (lower and upper whiskers).*
** In Figure D, ● represents the geometric mean.*
References:
Grey water: See Table 2.2.
Rain water: Holländer et al. (1996); Appan (1999); Simmons et al. (2001); Evans et al. (2006); Lye (2002); May and Prado (2006); Sazakli et al. (2007).
Untreated wastewater: From Kay et al. (2007)
Treated wastewater: Rose et al. (1996); Al-Nakshabandi et al. (1997); Fujioka et al. (1999); Bahri et al. (2001); Rose et al. (2001); Alonso et al. (2006); Kay et al. (2007).

2.2.2. Grey water

Grey water is broadly defined as all in-building wastewater streams with the exclusion of toilet wastewater. Further subdivision into 'light' and 'dark' streams is common; the former consisting of bathroom grey water from wash basins, baths, and showers; and the latter including grey water from the kitchen sink, dishwasher and laundry washing. The pollution loads in grey water are known to be highly variable (Jefferson *et al.* 2004) but dark grey water is typically more polluted (Friedler, 2004). To illustrate, COD values range from 100 to 645 $mg.L^{-1}$ in light grey water and from 361 to 1815 $mg.L^{-1}$ in dark grey water (Table 2.1.). At their lowest, the water quality parameters for grey water compare to a poor quality treated wastewater, and at their highest, they are comparable to untreated wastewater entering a sewage treatment facility. In respect to microbiological characteristics, the average concentrations of indicator bacteria in grey water are similar to those of secondary treated wastewater effluent, with median total coliform values of 4.7 and 4.8 $\log_{10}.100mL^{-1}$ for grey water and treated wastewater, respectively (Figure 2.1.). Data for indicator bacteria in grey water varies greatly though, with total coliform concentrations ranging from as low as 1.7 $\log_{10}.100mL^{-1}$ (Rose *et al.*, 1991; Dixon *et al.*, 1999) up to 8.8 $\log_{10}.100mL^{-1}$ (Gerba *et al.*, 1995). Faecal coliforms, *E. coli*, or Enterococci are reported in almost all of the literature studies and in all types of grey water stream (Table 2.2.), demonstrating that faecal contamination of grey water is not an occasional occurrence but is to be expected. Concentrations of faecal coliforms, *E. coli* and Enterococci in grey water are also comparable with those of secondary treated wastewater effluent, although slightly higher, with median values of 4.2, 3.3 and 2.8 $\log_{10}.100mL^{-1}$, respectively.

There is great variability in the concentrations of indicator bacteria reported in the literature for different grey water streams and between different studies examining similar grey water streams (Table 2.2.). Total coliform ranges reported for individual bathroom and laundry streams are similar at 1.7 to 5.8 $\log_{10}.100mL^{-1}$, whereas combined bathroom streams are generally higher at 2.7 to 7.4 $\log_{10}.100mL^{-1}$ (Christova-Boal *et al.*, 1996; Dixon *et al.*, 1999; Birks *et al.*, 2004).

Table 2.2. *Indicator bacteria in different grey water streams.*

Grey water source	Total coliforms ($log_{10}.100mL^{-1}$)	Faecal coliforms / Thermostable coliforms ($log_{10}.100mL^{-1}$)	Escherichia coli ($log_{10}.100mL^{-1}$)	Enterococci / Faecal Streptococci ($log_{10}.100mL^{-1}$)	Heterotrophic bacteria ($log_{10}.mL^{-1}$)	References
Wash Basin (WB)	4.7 – 5.8	1.5 – 3.5	3.8	>2.3	>5.5	Surendran and Wheatley (1998); Birks *et al.* (2004); Friedler (2004)
Shower (SH)	3.0 – 5.0	1.0 – 6.6	2.8 – 3.2	1.5 – 3.3	5.0 – 8.4	Rose *et al.* (1991); Nolde (1999); Friedler (2004); Jefferson *et al.* (2004)
Bath (BA)	1.7 – 4.4	6.6	1.9 – 4.3	1.0 – 1.6	ND	Dixon *et al.* (1999); Friedler (2004); Jefferson *et al.* (2004)
Light grey water - Mixed (WB, SH, or BA)	2.7 – 7.4	1.0 – 5.7	0.5 – 4.4	1.9 – 3.4	5.0 – 7.4	Christova-Boal *et al.* (1996); Surendran and Wheatley (1998); Nolde (1999); Casanova *et al.* (2001a); Eriksson *et al.* (2003); Jefferson *et al.* (2004); Friedler *et al.* (2005, 2006); Grant *et al.* (2006)
Laundry (LA)	1.7 – 5.8	1.4 – 6.6	ND	1.4 – 3.4	7.6 – 8.3	Rose *et al.* (1991); Christova-Boal *et al.* (1996); Surendran and Wheatley (1998); Dixon *et al.* (1999); Friedler (2004)
Kitchen sink (KS)	ND	6.1	ND	ND	ND	Friedler (2004)
Dishwasher (DW)	ND	4.8	ND	ND	ND	Friedler (2004)
Dark grey water - Mixed (WB, SH, BA, LA, KS, or DW)	7.2 – 8.8	4.9 – 7.9	2.0 – 6.0	2.4 – 4.6	ND	Gerba *et al.* (1995); Fittschen and Niemczynowicz (1997); Günther (2000); Casanova *et al.* (2001a, 2001b); Ottoson and Stenström (2003a); Dallas and Ho (2005); Gross *et al.* (2005)

Key: Greywater sources: BA=Bath, DW=Dishwasher, KS=Kitchen sink, LA=Laundry washing, SH=Shower, WB=Wash basin
ND = no data

The inclusion of wastewater from the kitchen sink significantly increases the levels of indicator bacteria in grey water (Casanova *et al.*, 2001a), with reported total coliform concentrations of up to 8.8 $\log_{10}.100mL^{-1}$ (Gerba *et al.*, 1995). Concentrations of the specific faecal indicators are also much greater, with Enterococci concentrations of up to 4.6 $\log_{10}.100mL^{-1}$, compared to a maximum of 3.4 $\log_{10}.100mL^{-1}$ reported for laundry or bathroom grey water (Christova-Boal *et al.*, 1996; Günther, 2000).

The pathogen population of grey water from the shower, bath, wash basin, and washing machine will include those pathogens colonising the body surface and orifices such as the nose and mouth. Faecal contamination of grey water is common, meaning that enteric pathogens may be present in grey water. Ottoson and Stenström (2003a) estimated the faecal load of grey water from all streams combined to be $0.04g.person^{-1}.day^{-1}$. Grey water from the kitchen sink may also contain enteric pathogens washed from raw meat or vegetables, which can harbour pathogens such as *Campylobacter* and *Salmonella* (Cogan *et al.*, 1999; Beuchat, 2002; Rose *et al.*, 2002). Despite the availability of microbiological data for a range of indicator bacteria, there is a shortage of reliable data on specific pathogens in grey water.

A small number of studies have tested for specific pathogens in grey water but many of these studies provide few details of the pathogen testing methodology, making it difficult to evaluate the meaning of the reported results, particularly in cases where no pathogens were detected. For instance, studies have reported testing negative for the presence of *E. coli* O157, *Salmonella* spp., *Shigella* spp., *Campylobacter* spp., and *Legionella pneumophilia* serotype 1, *Giardia* spp. and *Cryptosporidium* spp., in grey water (Christova-Boal *et al.*, 1996; Birks *et al.*, 2004; Birks and Hills, 2007). However, with no details provided regarding the maximum volume of grey water analysed, no information can be acquired about the maximum possible concentrations of these pathogens in the grey water samples tested.

Several common opportunistic pathogens have been reported in grey water (Table 2.3.). *P. aeruginosa* was reported by Casanova *et al.* (2001b) who analysed twenty grey water samples from a single household over a seven-month period for *P. aeruginosa* and

Staph. aureus. *P. aeruginosa* was present in every sample tested at a mean reported concentration of 4.3 $\log_{10}.100mL^{-1}$, while *Staph. aureus* was not detected in any of the samples. In contrast, Burrows *et al.* (1991) reported extremely high numbers of *Staph. aureus* in shower grey water from military camps, between 7.0 – 7.7 $\log_{10}.100mL^{-1}$. No details were given, however, of the testing methodology or whether these numbers represented confirmed or presumptive *Staph. aureus* colonies. In a recent study, confirmed *P. aeruginosa* and *Staph. aureus* bacteria were both reported in light grey water by Gilboa and Friedler (2007) at concentrations of 3.5 and 4.0 $\log_{10}.100mL^{-1}$, respectively. Environmental strains of *Legionella pneumophilia* (serotypes 2-14) have been recorded in wash basin grey water samples, collected in a large facility, at concentrations between 2.2 and 2.9 $\log_{10}.100mL^{-1}$, however, the strain most commonly associated with infections (serotype 1) was not detected (Birks *et al.*, 2004).

The faecally transmitted pathogens, *Salmonella* sp., *Giardia*, and *Cryptosporidium* have been detected in grey water, at concentrations of up to 7.9 and 8.3 $\log_{10}.100L^{-1}$ for *Giardia*, and *Cryptosporidium*, respectively (Birks *et al.*, 2004; Birks and Hills, 2007). The detection of these pathogens in grey water requires an affected individual contributing to the grey water tested, which is more probable with a larger contributing population, like the one studied by Birks *et al.* (2004). However, the grey water produced by a larger contributing population will dilute enteric pathogens, resulting in lower concentrations and requiring sampling of large quantities of grey water for pathogen detection. Higher concentrations of specific pathogens in grey water are more likely to occur in smaller reuse schemes due to a lower dilution effect. The demographic of the contributing population has also been shown to affect the microbiological quality of grey water. Households with young children have been shown to produce grey water with higher levels of total and faecal coliforms than households with no children (Rose *et al.*, 1991; Casanova *et al.*, 2001a). For example, Rose *et al.* (1991) reported average total coliform concentrations of 5.5 $\log_{10}.100mL^{-1}$ in grey water produced by families with young children, compared to around 2 $\log_{10}.100mL^{-1}$ for households with no children. A further important issue is whether pathogens in grey water regrow or die-off during storage time. Coliform bacteria in stored grey water have been shown to increase in concentration by as much as 2 $\log_{10}$ units within 24 or 48 hours (Rose *et al.*, 1991;

Dixon *et al.*, 1999), demonstrating the impact storage time can have on indicator bacteria in grey water. In contrast, the enteric pathogenic bacteria, *Salmonella* sp., *Shigella* sp., and *Campylobacter* sp., do not appear to regrow in grey water (Rose *et al.*, 1991; Ottoson and Stenström (2003b), although *Salmonella* sp., *Shigella* sp., and poliovirus have been shown to persist in grey water for a matter of days (Rose *et al.*, 1991).

Table 2.3. *Pathogens reported in urban waters.*

Potential pathogen	Urban water	Concentration (log_{10})	Reference
Aeromonas spp. (.100mL^{-1})	Rain water	1.0 – 1.5	Albrechtsen (2002)
	Rain water	NR	Simmons *et al.* (2001)
Pseudomonas aeruginosa (.100mL^{-1})	Grey water	4.3	Casanova *et al.* (2001b)
	Grey water	3.5	Gilboa and Friedler (2007)
	Rain water	0 – 1.3	Albrechtsen (2002)
	Rain water	1.9	Holländer *et al.* (1996)
	Rain water	0.6 – 1.5	May and Prado (2006)
	Rain water	NR	Adeniyi and Olabanji (2005)
Staphylococcus aureus (.100mL^{-1})	Grey water	4.0	Gilboa and Friedler (2007)
Legionella pneumophilia (.100mL^{-1})	Grey water	2.2 – 2.9	Birks *et al.* (2004)
	Rain water	NR	Schlech *et al.* (1985)
Mycobacterium spp. (.100mL^{-1})	Rain water	NR	Albrechtsen (2002)
	Rain water	NR	Tuffley and Holbeche (1980)
Campylobacter (.100mL^{-1})	Rain water	NR	Albrechtsen (2002)
Salmonella spp. (.100mL^{-1})	Grey water	NR	Birks and Hills (2007)
	Rain water	NR	Simmons *et al.* (2001)
	Rain water	NR	Holländer *et al.* (1996)
Cryptosporidium spp. (.100L^{-1})	Grey water	0 – 8.3	Birks *et al.* (2004)
	Rain water	0 – 3.7	Albrechtsen (2002)
	Rain water	0 – 1.0	Crabtree *et al.* (1996)
	Rain water	NR	Simmons *et al.* (2001)
Giardia spp. (.100L^{-1})	Grey water	0 – 7.9	Birks *et al.* (2004)
	Grey water	1.7 – 2.2	Birks and Hills (2007)
	Rain water	0 – 1.3	Birks *et al.* (2004)
	Rain water	0 – 1.0	Crabtree *et al.* (1996)

NR: Not reported

2.2.3. Rain water

The collection and reuse of rain water is a common practice throughout the world and is used as a source of drinking water in some areas (Lye, 1992; Simmons *et al.*, 2001). Harvesting of rain water for reuse typically involves collection via a rooftop catchment area followed by storage in an appropriate container.

The water quality of rain water is generally very good in comparison to grey water, with BOD values of less than 3.7 $mg.L^{-1}$, although relatively high levels of suspended solids and turbidity, up to 153 $mg.L^{-1}$ and 56 NTU, respectively, have been reported in rain water (Table 2.1.). The chemical, physical, and microbiological quality of collected rain water is largely determined by environmental factors. Access to birds, small mammals, and even insects can result in faecal contamination and passage of pathogenic microorganisms and parasitic organisms into rain water (Lye, 2002). Airborne microorganisms can also contaminate collected rain water and the degree to which this occurs is influenced by wind velocity and direction (Evans *et al.*, 2006). Roof material type and age can also impact bacterial counts of roof-collected rain water (Adeniyi and Olabanji, 2005). For instance, lower numbers of bacteria were recorded in storage tanks made from manufactured plastic than those constructed from concrete or bricks in a study of 102 rain water cisterns by Holländer *et al.* (1996). Sazakli *et al.* (2007) observed seasonal variation in the microbial quality of collected rain water, reporting the highest number of samples positive for indicator bacteria at the start of the rainy season, and concluded that this was due to the preceding dry season, when catchment surfaces become contaminated without wash-off.

Total coliform bacteria are regularly reported in rain water, at concentrations up to 4.3 $\log_{10}.100mL^{-1}$ (Figure 2.1.). However, the maximum concentrations of total and faecal coliforms reported in rain water are lower than the average median values for grey water and treated wastewater. Faecal contamination of harvested rain water is common. For example, 41% of rain water samples tested by Sazakli *et al.* (2007) in Kefalonia, Greece, were positive for *E. coli*. Similarly, sampling of a rooftop rain water collection system in São Paulo, Brazil, revealed faecal coliforms in 50% of samples and Enterococci in 98% of the samples tested (May and Prado, 2006). Specific faecal pathogens have also been reported in rain water (Table 2.3.). *Campylobacter* and *Salmonella* spp. have been detected in harvested rain water, as well as the protozoa *Cryptosporidium* and *Giardia*, at concentrations up to 3.7 $\log_{10}.100L^{-1}$. Detections of *Salmonella* spp. in rain water are rare, however, and were detected in just 0.9% and 0.1% of rain water samples tested in New Zealand (Simmons *et al.*, 2001) and Germany (Holländer *et al.*, 1996). *Campylobacter* has been detected in rain water by Albrechsten

(2002) and, in New Zealand; cases of diarrhoeal disease caused by *Campylobacter* spp. have been linked to potable rain water collection systems (Eberhart-Phillips *et al.*, 1997).

A number of opportunistic pathogens have also been reported in rain water. The most commonly detected is *P. aeruginosa*, at concentrations up to 1.9 $\log_{10}.100mL^{-1}$ (Holländer *et al.*, 1996). Other detected pathogens include *Mycobacterium* spp. (Tuffley and Holbeche, 1980; Albrechtsen, 2002), *Aeromonas* spp. (Simmons *et al.*, 2001), and *Legionella pneumophilia* (Schlech *et al.*, 1985). The two latter pathogens were linked to gastrointestinal illness and Legionnaires' disease, respectively, amongst users of the rain water as a potable water supply.

2.2.4 Implications for urban water disinfection

The urban waters, black water, grey water, and rain water, all have the potential to harbour pathogenic microorganisms. Although specific pathogenic viruses have not been reported in grey water or rain water, the occurrence of bacterial and protozoan pathogens means that the presence of viral pathogens can be anticipated. For all urban waters, disinfection technologies must therefore be capable of removing or inactivating bacteria, protozoa, and viruses; if pathogen-free water is desired for urban reuse. The concentrations of pathogens differ between urban waters, as do the chemical/physical water quality parameters, with declining overall water quality from rain to grey to black water. The chemical and physical water quality, particularly organic and particulate material, strongly impact the efficacy of disinfection processes and, as such, will also influence the selection of appropriate disinfection technology for urban water reuse.

Potential disinfection technologies for reuse are reviewed with regard to their pathogen elimination performance and the impact of water quality on the efficacy of disinfection. Use of the technologies to disinfect urban waters and their real-world application for water reuse are also reported.

2.3. DISINFECTION OPTIONS FOR URBAN WATER REUSE

Disinfection is the process of eliminating pathogenic organisms in water and is achieved by their inactivation in the water or their physical removal from the water. Although pathogenic microorganisms are the targets of disinfection, it is indicator bacteria that are most commonly used to assess the microbial quality of the disinfected effluent. This is because the detection and enumeration of all pathogens of concern in waters is both a time-consuming and costly process, and unfeasible for routine analysis. Total and faecal coliforms, *Escherichia coli*, and Enterococci are the most commonly used indicator bacteria. Spore-forming bacteria, such as *Clostridia* spp., or *Bacillus* spp.; and bacteriophage, such as coliphages, are also used, often as indicators for protozoan and viral pathogens. Indicator microorganisms are not consistently reliable indicators of specific pathogens (Leclerc *et al.*, 2001) but their inclusion in numerous water reuse standards worldwide (USEPA, 2004) stipulates their importance as indicators for the disinfection of water for reuse.

Disinfection technologies can be broadly categorised as chemical- and physical-based systems. Principal chemical disinfectants include the halogens, of which chlorine is the predominant form, and ozone. Plant essential oils are also investigated in this review as potential novel chemical disinfectants for water. Physical disinfection technologies include those that operate by the input of energy into the water, such as ultraviolet (UV) light and ultrasound energy, and those that physically separate microorganisms from the water, by filtration through media or a membrane.

Microorganism inactivation by disinfection processes are influenced by the characteristics of the water to which the disinfection is applied. These characteristics include: the specific microorganisms present, temperature, pH, chemical constituents (e.g. organic or inorganic matter), and physical constituents (e.g. particulate material). These water characteristics do not equally affect disinfection processes meaning that some disinfection technologies are more suited for the disinfection of certain water types than others. The aforementioned disinfection technologies are reviewed here, with emphasis on the impact of organic and particulate material on the efficacy of

disinfection. Their application for the disinfection of urban waters, principally grey water, as well as treated wastewater destined for reuse is also reviewed and discussed.

2.3.1. Chemical disinfection

Chemical disinfection involves the addition of a compound to water that is toxic to microorganisms, causing their inactivation. The most common chemical disinfectants are chlorine and ozone, which are highly oxidising chemicals and are thought to cause microbial inactivation by direct oxidation of microorganisms, causing damage to cell membranes, DNA, proteins, and enzymes (Asano *et al.*, 2007). The degree of microbial inactivation is determined by the applied disinfectant dose, which is a function of both the chemical concentration and the time of exposure (Chick, 1908; Watson, 1908). Chemical disinfectant doses are therefore characterised by the *Ct* concept, which represents the disinfectant concentration multiplied by the contact time ($mg.min.L^{-1}$).

The efficacy of chemical disinfection is affected by the quality of the water to which the disinfectant is applied. Organic or inorganic material can react with the disinfectant, effectively deactivating it and preventing its use for the inactivation of microorganisms. This is referred to as disinfectant 'demand' and is responsible for a lag phase (Figure 2.2.), which is typically observed when chemical disinfectants are applied to waters. Once the disinfectant dose exceeds the demand, linear inactivation of microorganisms is observed, with increasing disinfectant dose resulting in a proportional inactivation of the dispersed targeted microorganisms, according to the Chick-Watson rate law (Haas and Karra, 1984). Particulate material in water can impact chemical disinfection by shielding microorganisms embedded within particles or aggregates from the applied disinfectant. The presence of particle-associated or aggregated microorganisms results in a deviation from the Chick-Watson rate law and is indicated by a tailing phase, where the remaining concentration of particle-shielded microorganisms is effectively independent of the disinfectant dose (Figure 2.2.).

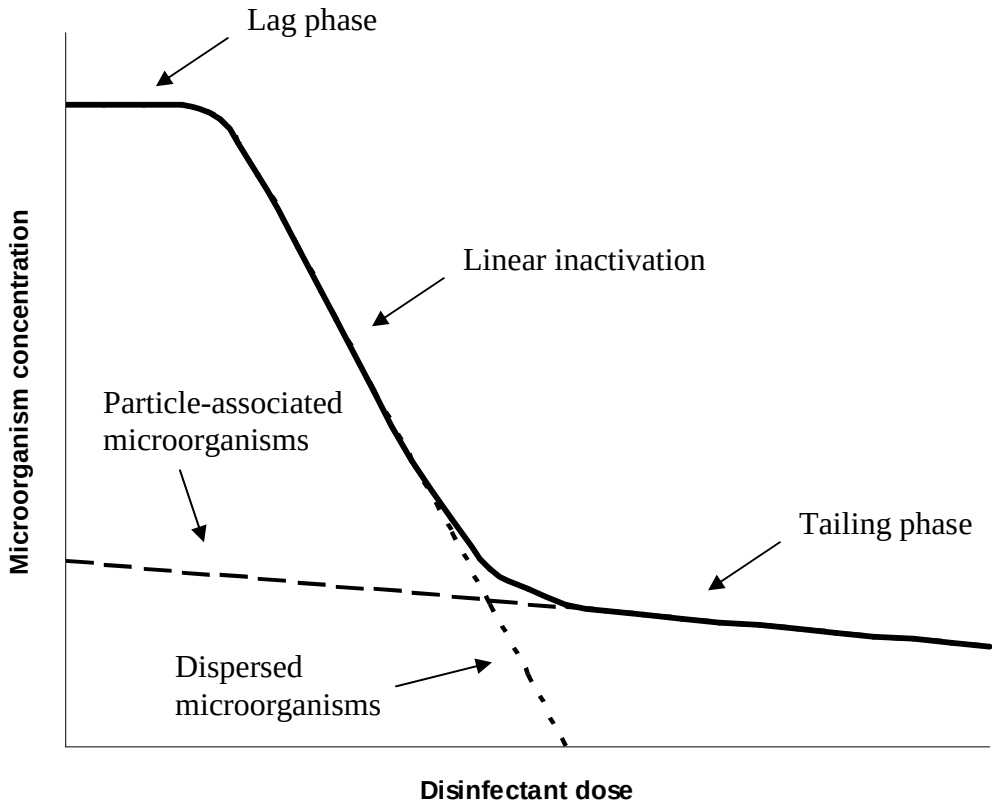

Figure 2.2. *Example microorganism inactivation curve with increasing disinfectant dose showing all microorganisms (solid line), and the dispersed or particle-associated fractions. Lag, linear inactivation, and tailing phases are indicated. Adapted from Emerick et al. (2000) and Asano et al. (2007).*

2.3.1.1. Chlorine

Chlorine is used in a number of forms to provide disinfection of wastewater. These include chlorine gas or liquid (Cl_2), sodium or calcium hypochlorite (NaOCl or $Ca(OCl)_2$), chlorine dioxide (ClO_2), and chloramines (primarily NH_2Cl and $NHCl_2$). Chlorine (Cl_2) and chlorine dioxide are unlikely to be suitable disinfectants for the decentralised reuse of urban waters due to safety and cost concerns regarding their generation, handling, and storage. For the purposes of reuse, sodium or calcium hypochlorite are the most suitable because free chlorine (as HOCl) typically requires a lower concentration and a shorter contact time to provide an equivalent level of microbial inactivation as chloramines (Tchobanoglous *et al.*, 2003). One significant advantage of calcium hypochlorite is its availability in solid form, as compressed pellets

or tablets, making it easier to handle and particularly suitable for smaller-scale reuse schemes.

The specific mechanism of microorganism inactivation by chlorine compounds is not fully understood. Studies have shown that the permeability of the bacterial membrane is affected in the presence of chlorine and that the stability of the membrane determines bacterial resistance to chlorine disinfection (Venkobachar *et al.*, 1977; Virto *et al.*, 2005). In viruses chlorine has been shown to cause inactivation by damaging the viral genome and capsid proteins (Li *et al.*, 2002; Li *et al.*, 2004).

Typical initial chlorine dosages for wastewater applications range between 60 and 900 $mg.min.L^{-1}$, respectively (Table 2.4.). Susceptibility to chlorine disinfection varies greatly between different types of pathogenic microorganisms (Table 2.4.) with bacteria the most susceptible, followed by viruses, and then protozoa. *Ct* values required for a 3 log_{10} inactivation of different bacteria varied from $0.03mg.min.L^{-1}$ for *E. coli* to $43mg.min.L^{-1}$ for *Mycobacterium aurum* (Helbling and VanBriesen, 2007). Low *Ct* values have also been shown to give 4 log_{10} or greater virus inactivation, with reported *Ct* values of $0.22mg.min.L^{-1}$ for adenovirus and $1.5mg.min.L^{-1}$ for a surrogate norovirus (feline calicivirus) (Baxter *et al.*, 2007; Urakami *et al.*, 2007). However, chlorine is less effective for the inactivation of pathogenic protozoa, with reported *Ct* values of between 3700 and 7200 $mg.min.L^{-1}$ providing just 2 log_{10} inactivation of *Cryptosporidium parvum* oocysts (Korich *et al.*, 1990; Driedger *et al.*, 2000).

Water quality strongly influences the efficacy of disinfection by free chlorine (as HOCl). Free chlorine reacts with inorganic and organic substances in water, reducing the availability of free chlorine for disinfection and resulting in a lag phase (Figure 2.2.). Inorganic substances, such as ammonia (NH_3) and iron (Fe^{2+}) react rapidly with chlorine (Fair *et al.*, 1948), creating an instant chlorine demand. Free chlorine reaction with organic substances, in contrast, is more variable and comparatively slow (Hendricks, 2006; Warton *et al.*, 2006). Highly polluted waters in terms of organics necessitate the use of high initial chlorine concentrations to surpass the initial chlorine

demand and to compensate for chlorine decay. Indeed, March *et al.* (2004) observed the decay of up to 36 $mg.L^{-1}$ chlorine in grey water over a 12 hour period.

Microbial aggregates or microorganisms associated with particulate matter in water have greater resistance to inactivation by chlorine compared to dispersed microorganisms (Hejkal *et al.*, 1979; Bohrerova and Linden, 2006). For instance, LeChevallier *et al.* (1981) showed the shielding of bacteria within particles in potable water by scanning electron microscopy and reported a five-fold increase in numbers of heterotrophic bacteria following the extraction of particle-associated bacteria. Particle size also affects the shielding of microorganisms from chlorine. Wastewater particles >7 µm in size have been shown to reduce the inactivation rate of coliform bacteria exposed to free chlorine (Berman *et al.*, 1988), and the resistance of coliforms to chlorine has been shown to increase with increasing particle size in wastewater (Madge and Jensen, 2006). The increased resistance of particle-associated microorganisms to chlorine disinfection is responsible for the tailing phase of disinfection (Figure 2.2.), which can prevent compliance with water reuse standards.

Chlorine has been shown to be effective for the disinfection of waters for reuse. For instance, Rose *et al.* (1996) reported log_{10} inactivations of 4.2, 1.5, and 0.7 for total coliforms, enteroviruses, and *Cryptosporidium*, respectively, in tertiary treated wastewater destined for use as irrigation water. March *et al.* (2004) reported zero detection of total coliforms following the application of 75 $mg.L^{-1}$ initial chlorine to coarsely filtered grey water destined for reuse in hotel toilets. The high chlorine concentration achieved penetration of particulate material (Dietrich *et al.*, 2003) and was required to maintain a free chlorine residual of above 1 $mg.L^{-1}$, below which, bacterial regrowth was observed (March *et al*, 2004). Friedler *et al.* (2006) applied chlorine disinfection (~42-84$mg.min.L^{-1}$) to treated grey water effluents of variable quality, observing complete inactivation faecal coliforms and no regrowth for up to one week of storage. Regrowth of heterotrophic bacteria was observed after one week, however, when chlorine residuals had fallen to <0.3 $mg.L^{-1}$. Heterotrophic regrowth was greatest in the poorest quality grey water effluent.

Table 2.4. *Overview of disinfection processes.*

Disinfection process		Chemical			Physical - Energy		Physical - Filtration	
		Chlorine	Ozone	Essential oils	UV	Ultrasound	Media	Membrane
Units		$mg.min.L^{-1}$	$mg.min.L^{-1}$	$\mu L.L^{-1}$	$mJ.cm^{-2}$	$J.L^{-1}$	n/a	n/a
Target for microbial inactivation		Bacterial membrane, viral capsid	Bacterial membrane, DNA, viral capsid	Bacterial membrane	DNA	Bacterial membrane, DNA	Whole microorganism	Whole microorganism
Organic sensitivity		Yes	Yes	Yes	Yes	No	Yes	No
Particle sensitivity		Yes	Yes	Unknown	Yes	No	No	No
Residual disinfection		Yes	No	Yes	No	No	No	No
Typical disinfectant dose for 2-log inactivation of:	Bacteria	0.4 – 0.6	3 – 4	-	30 – 60	-	-	-
	Protozoa	60 – 70	0.9 – 1.2	-	10 – 20	-	-	-
	Viruses	1 – 4	0.4 – 0.6	-	30 – 40	-	-	
Typical total coliform log removal for secondary wastewater effluent		-	-	-	-	-	0 – 1	2 – >4
Typical initial dosage for secondary activated sludge effluent to achieve total coliform ($.100mL^{-1}$) levels:	1000	60 – 300	60 – 120	-	40 – 50	-	-	-
	200	150 – 450	60 – 150	-	50 – 70	-	-	-
	23	300 – 900	240 – 450	-	70 – 90	-	-	-
	≤2.2	-	450 – 600	-	90 – 110	-	-	-
Typical initial concentration for potable water disinfection		1 – 6 $mg.L^{-1}$	1 – 5 $mg.L^{-1}$	-	20 – 100	-	-	-

Adapted from Tchobanoglous et al. (2003); Crittenden et al. (2005); Asano et al. (2007).

2.3.1.2. Ozone

Ozone is triatomic oxygen (O_3) and is typically produced by introducing oxygen gas with an electric discharge, splitting oxygen molecules into atoms, which then combine with the remaining oxygen molecules to form ozone. Ozone is highly reactive and its use in water leads to the production of hydroxyl radical (OH·) species, which are powerful, non-specific oxidants (Haag and Yao, 1992). Ozone will react with bacterial cell components, including DNA (Ishizaki *et al.*, 1987), to cause inactivation and has been shown to cause inactivation of viruses by breaking the protein capsid and disrupting adsorption to host cells (Kim *et al.*, 1980). The high oxidative power of ozone means that, as well as providing disinfection, it can also significantly improve the overall quality of wastewater by oxidising organics and reducing turbidity, odour, and colour (Paraskeva *et al.*, 1998; Pokhrel and Viraraghavan, 2004).

Initial ozone doses typically applied in wastewater applications range from 60 to 600 $mg.min.L^{-1}$ (Table 2.4.). Ozone is particularly effective for the inactivation of resistant microorganisms such as protozoan cysts and viruses, providing a 2 log_{10} inactivation with a dose of 0.4-1.2 $mg.min.L^{-1}$. For a greater 4 log_{10} inactivation (at 5°C), *Ct* values of <0.03, <0.60, and 61 $mg.min.L^{-1}$ have been reported, for feline calicivirus, adenovirus, and *Cryptosporidium parvum* oocysts, respectively (Rennecker *et al.*, 1999; Thurston-Enriquez *et al.*, 2005). Ozonation of river water seeded with microorganisms provided up to 4 log_{10} inactivation of total coliform bacteria at a *Ct* dose of $6.3mg.min.L^{-1}$ and more than 2 log_{10} inactivations of *Cryptosporidium, Giardia,* and poliovirus at *Ct* values of less than $10mg.min.L^{-1}$ (Owens *et al.*, 2000). Low *Ct* inactivation doses for ozone are due to the short contact times typically required for microbial inactivation compared to other chemical disinfectants, such as chlorine (Tyrrell *et al.*, 1995). Indeed, increasing ozone contact time from 2 to 10 minutes did not improve the inactivation of coliform bacteria in wastewater effluents (Xu, *et al.*, 2002).

Disinfection with ozone is impacted by the presence of organic and particulate material in water. Organics in water can encourage the decomposition of ozone by promoting a

chain reaction of decomposition (Staehelln and Hoigne, 1985; Pi *et al.*, 2005), reducing the available ozone and free OH· radicals available for disinfection. Studies have shown reduced ozone inactivation rates of bacteria in the presence of organic material and attributed this to the increased ozone demand created by the organics (Restaino *et al.*, 1995; Hunt and Mariñas, 1999). However, significant inactivation of up to 3 $\log_{10}$ coliform bacteria can occur before the ozone demand is met because bacteria themselves participate in the ozone demand of the disinfected water (Janex *et al.*, 2000; Xu *et al.*, 2002). The ability of ozone to inactivate particle-associated microorganisms is limited by ozone demand which must first be met before ozone diffusion into particles will occur (Dietrich *et al.*, 2007). Filtration to remove suspended solids from wastewater effluent has been shown to reduce required ozone doses and allow treatment to a standard for reuse (Janex *et al.*, 2000; Xu, *et al.*, 2002).

An important concern with ozone disinfection is the potential for bacterial regrowth following disinfection. Ozone decays with time (Paraskeva *et al.*, 1998) and upon reaction with impurities in the water, leaving no residual disinfectant. In addition, ozone can increase the bioavailability of organic matter in wastewater as a result of the oxidisation of previously non-biodegradable compounds (Xu, *et al.*, 2002) and can also increase the microbially available phosphorous, increasing the regrowth potential of heterotrophic microbes (Lehtola *et al.*, 2001).

The application of ozone for the disinfection of wastewaters intended for reuse with toilet flushing, laundry, and garden irrigation has been reported in the literature (Townshend *et al.*, 1997; Seo *et al.*, 2001; Petala *et al.*, 2006). Filtration to remove suspended solids from wastewater effluent is typically required to meet stringent reuse standards. For instance, Xu *et al.* (2002) reported an additional 1 $\log_{10}$ inactivation of total coliforms by ozone following 10 µm filtration of wastewater effluent. Janex *et al.* (2000) reported that ozone doses of 15mg.min.L^{-1} applied to filtered secondary wastewater effluent would meet the Title 22 standards for water reuse ($\leq$2.2 coliforms.100mL^{-1}) compared to more than 80mg.min.L^{-1} applied to unfiltered effluent. Ozone concentrations of 7.1 and 26.7 mg.L^{-1} applied to tertiary treated wastewater effluent provided total coliform concentrations of 240 and <2 per 100mL, respectively,

the latter meeting the USEPA and Title 22 requirements for urban water reuse (Petala *et al.*, 2006).

2.3.1.3. Essential oils

Essential oils are aromatic, oily liquids extracted from plant material, such as seeds, leaves and flowers, by steam distillation or other methods (Sangwan *et al.*, 2001). Many essential oils exert antimicrobial activity (Hammer at al., 1999), which is typically strongest in those essential oils with phenolic components, such as carvacrol, thymol, or eugenol (Cosentino *et al.*, 1999; Lambert *et al.*, 2001; Peñalver *et al.*, 2005; Soković and van Griensven, 2006). Despite the complex composition of essential oils, the principal mechanism of bacterial inactivation by essential oils appears to be the disruption of the cell membrane, resulting in cell leakage and eventual cell lysis (Burt, 2004). Support for this mechanism has been provided by scanning electron microscope images of porous and flaccid *E. coli* cells following exposure to origanum essential oil (Burt and Reinders, 2003; Rhayour *et al.*, 2003). Essential oils have also been shown to exert antiprotozoan (Anthony *et al.*, 2005; De Almeida *et al.*, 2007) and antiviral (Schuhmacher *et al.*, 2003; Edris, 2007) activity. The use of essential oils for water disinfection has received little attention but their application as food preservatives is currently the focus of much research (Burt, 2004).

Numerous different methods have been used to assess the antibacterial qualities of essential oils on pure cultures. A minimum inhibitory concentration (MIC) is most typically reported, however, this can vary with the method used and with the addition of chemicals to disperse oils (Remmal *et al.*, 1993; Burt and Reinders, 2003). Nevertheless, Hammer *et al.* (1999) tested 52 plant essential oils and reported MICs from 600 $\mu L.L^{-1}$ for the bacteria *E. coli* and *Staph. aureus*, 1200 $\mu L.L^{-1}$ for *Salmonella* sp. and 10000 $\mu L.L^{-1}$ for *P. aeruginosa*. MICs for origanum essential oil are typically some of the lowest compared to other essential oils. To illustrate, origanum oil MICs of 625, 575, and 1648 $\mu L.L^{-1}$ have also been reported for *E. coli*, *Staph. aureus* and *P. aeruginosa*, respectively (Lambert *et al.*, 2001; Burt and Reinders, 2003). Essential oils have also been shown to be effective in the inactivation of parasitic microorganisms,

including the protozoan *Giardia lamblia* (Anthony *et al.*, 2005). For instance, the essential oil of *Ocimum basilicum* provided 80% inactivation of *Giardia lamblia* trophozoites at a concentration of 2000mg/L and after two hours contact time (de Almeida *et al.*, 2007).

While essential oils have been shown to inhibit bacterial growth *in vitro*, greater concentrations are generally required to achieve similar levels of inhibition in foods (Burt, 2004). Organic sources, such as fats or proteins in foods or oil stabilisers, can reduce the antimicrobial effect of essential oils (Juven *et al.*, 1994; Tassou *et al.*, 1995; Singh *et al.*, 2003), indicating that organic matter in urban waters is likely to impact disinfection by essential oils. There are conflicting reports regarding the effects of temperature on the antibacterial properties of essential oils. Studies have reported no effect of temperature (Smith-Palmer *et al.*, 1998; Burt and Reinders, 2003), a reduction in antibacterial activity at lower temperatures (Smith-Palmer *et al.*, 1998), and an increase in antibacterial activity at lower temperatures (Burt, 2004). Low pH has been shown to increase the antibacterial activity of essential oils (Juven *et al.*, 1994; Burt, 2004).

A key concern regarding the use of essential oils for urban water disinfection is maintaining the dispersion of the essential oil throughout the liquid. The addition of solvents or detergents, such as ethanol or Tween-80, creates a homogenous solution but they have been shown to reduce the antimicrobial properties of essential oils (Remmal *et al.*, 1993). The use of low agar concentrations (<0.2% w/v)) to disperse essential oils in water is an effective solution (Remmal *et al.*, 1993) but the practicality of using agar for the disinfection of urban waters for reuse would require further investigation.

2.3.2. Physical disinfection

Physical disinfection processes involve either the addition of energy to water to bring about microbial inactivation, as is the case with ultraviolet light and ultrasound, or the mechanical separation of microorganisms from the water by filtration though media or a membrane. Energy-based disinfection by UV light and ultrasound can be quantified in

dose terms, whereas the efficacy of filtration processes is determined by operating conditions, which are highly variable, particularly for media filtration. The water quality, specifically organic and particulate material, also impacts most physical disinfection processes but in different ways to chemical disinfection processes.

2.3.2.1. Ultraviolet (UV) light

UV light is electromagnetic radiation, which disinfects water by damaging the genetic material of microorganisms. Lesions in the form of pyrimidine dimers are induced in the genomic DNA or RNA of microorganisms (Gurzadyan *et al.*, 1995; Sinha and Häder, 2002), preventing normal replication, which effectively inactivates exposed microorganisms. UV light of interest for disinfection has a wavelength of between 220 and 320nm and is largely short-wave, or UV-C radiation. Medium pressure UV lamps produce a broad range of radiation within this range, while low pressure UV lamps emit UV light at a single wavelength of 254nm. The applied UV dose (or fluence) is traditionally characterised in terms of the energy per surface area, or $mJ.cm^{-2}$, and is a product of the average UV intensity (fluence rate) ($mW.cm^{-2}$) multiplied by the exposure time in seconds. UV is attenuated by UV-absorbing substances in water, which reduce the transmittance of UV through water and consequently impact the dose received by microorganisms. The UV transmittance of water to be disinfected is therefore taken into account when calculating the average UV intensity applied to water.

Typical UV doses for wastewater applications range from 40 to 110 $mJ.cm^{-2}$ with doses of up to 60 $mJ.cm^{-2}$ providing up to 2 $\log_{10}$ inactivations of most bacteria, viruses, and protozoa (Table 2.4.). A UV dose of 30 $mJ.cm^{-2}$ provides 3 $\log_{10}$ or greater inactivation of many important bacterial, protozoan and viral waterborne pathogens, including *E. coli* and *Salmonella* sp., *Giardia* and *Cryptosporidium*, poliovirus and hepatitis A virus (Hijnen *et al.*, 2006). However, certain microorganisms are more resistant to inactivation by UV, including adenoviruses and bacterial spores, which require high doses of up to 167 $mJ.cm^{-2}$ for a 3 $\log_{10}$ inactivation (Hijnen *et al.*, 2006).

Constituents in urban waters can impact the efficacy of disinfection with UV. The presence of UV-absorbing substances such as organic material and iron, reduce the dose received by targeted microorganisms by absorbing the UV light and reducing UV transmittance through the water (Asano *et al.*, 2007). The typical UV transmittance of secondary wastewater effluent is 45-70%, for example (Asano *et al.*, 2007). This can be overcome, however, by simply increasing the applied UV dose to compensate for the reduced UV transmittance (Havelaar *et al.*, 1990). The UV dose is raised by either increasing the light intensity, or by increasing the exposure time, which is achieved by reducing the flow of water through the UV reactor.

UV disinfection is greatly impacted by the presence of particles in water, which cause a tailing phase of disinfection, in a similar manner to chemical disinfection (Figure 2.2.). Particles can contain embedded microorganisms that are shielded from the applied UV light and therefore survive disinfection (Qualls *et al.*, 1983). It is the presence of microorganisms in regions inaccessible to UV light within particles that facilitates shielding from disinfection. Microorganism size influences the particle size capable of providing shielding from UV light. To illustrate, particles of around 10 µm will shield bacteria from UV light (Emerick *et al.*, 1999), whereas particles of <2 µm protect viruses (Templeton *et al.*, 2005), and particle sizes of more than 25 µm are considered necessary to shield protozoa from disinfection by UV light (Amoah *et al.*, 2005). Larger particle size (Madge and Jensen, 2006), low particle porosity (Loge *et al.*, 1999), and high UV-absorbing properties of particles (Templeton *et al.*, 2005; 2006) all increase the protection afforded to microorganisms by particulate matter in water. Further, larger particles in secondary wastewater effluents have been shown to harbour significant numbers of coliform bacteria following UV disinfection, with coliform counts up to 340 times greater following blending to release particle-associated microorganisms (Parker and Darby, 1995). Increasing UV exposure time may increase the probability of microorganism exposure to UV through light pathways within particles (Mamane and Linden, 2006), however, microorganisms in locations within particles to which no available light pathway exists, will receive no exposure to UV light and will therefore persist following disinfection. To ensure these deeply-embedded microorganisms do not

persist in disinfected effluents, the particles must be either removed, or sheared to disperse the microorganisms, prior to UV exposure.

A UV dose of 25-40 $mJ.cm^{-2}$ applied to biologically treated grey water effluent has been reported to reduce the coliform concentrations to within the Germany quality guidelines for reuse (4.0 $\log_{10}.100mL^{-1}$), however, significant numbers of total coliforms remained in the disinfected effluent, up to 2.7 $\log_{10}.100mL^{-1}$ (Nolde *et al.*, 1999). Similarly, Gilboa and Friedler (2007) recently investigated the UV disinfection of grey water treated by rotating biological contactor and observed no regrowth of faecal coliforms, *P. aeruginosa* or *Staph. aureus* up to 6 hours after exposure to UV doses from 0 to 439 $mJ.cm^{-2}$. The regrowth of heterotrophic bacteria was reported after UV doses of >147 $mJ.cm^{-2}$. At doses of up to 69 $mJ.cm^{-2}$, faecal coliform bacteria were found to be more resistant to inactivation than *P. aeruginosa* or *Staph. aureus* bacteria and this was attributed to the aggregation of faecal coliforms in the treated grey water effluent. The filtration of water to remove particles and microbial aggregates can significantly reduce the required UV dose required to disinfect to reuse standards (Jolis *et al.*, 2001). For instance, in a study by Darby *et al.* (1993), disinfection of secondary wastewater effluent with UV doses of up to 239 $mJ.cm^{-2}$ did not reach the California Title 22 standard for unrestricted urban reuse (0.5 $\log_{10}.100mL^{-1}$), however, following filtration of the wastewater effluent, the standard was consistently met with a UV dose of 97 $mJ.cm^{-2}$.

2.3.2.2. Ultrasound

Ultrasound is high frequency sound, above 20kHz, which when applied to water, induces cavitation (the formation and collapse of cavities within a liquid), creating regions of high pressure and temperature. Microbial inactivation is caused by the physical disruption of cells, the production of heat, and the formation of free radicals (Ahn *et al.*, 2003; Piyasena *et al.*, 2003). Madge and Jensen (2002) investigated the use of ultrasound for wastewater disinfection and demonstrated that 52% of the inactivation of faecal coliform bacteria could be attributed to thermal disinfection, and 36% to the

mechanical disruption of cells from cavitation. Microbial inactivation by ultrasound is by cell lysis (Kawai and Iino, 2003) and damage to DNA (Elsner and Lindblad, 1989).

Ultrasound can inactivate a range of bacterial pathogens, including *E. coli* and *Salmonella* spp. in a variety of liquids (Piyasena *et al.*, 2003); however ultrasound apparatus and operating conditions used vary considerably, making direct comparisons of performance difficult. A 5.1 $\log_{10}$ inactivation of pure culture *E. coli* was reported at an ultrasound dose of 3.2×10^{5} $J.L^{-1}$ by Ince and Belen (2001), whereas in wastewater effluent, a higher dose of 1.4×10^{6} $J.L^{-1}$ gave just 2.9 $\log_{10}$ inactivation of *E. coli* (Neis and Blume, 2003). With an even higher dose, 2.5×10^{6} $J.L^{-1}$, ultrasound achieved almost 2 $\log_{10}$ inactivation of Cryptosporidium parvum oocysts (Oyane *et al.*, 2005). The efficacy of ultrasound disinfection for viruses appears to vary with virus type (Scherba *et al.*, 1991). An investigation into the effect of wastewater quality on ultrasound disinfection revealed no significant impact of parameters including TSS, BOD, TOC, pH, and initial faecal coliform concentration, on the efficacy of disinfection (Madge and Jensen, 2002). TSS values as high as 600 $mg.L^{-1}$ were required to slightly decrease the rate of disinfection.

Ultrasound may be a suitable alternative disinfection technology for low quality waters where disinfection by chlorine or UV is impractical due to high organics and suspended solids. The main drawbacks of disinfection by ultrasound include the high energy input required for effective disinfection and the high temperatures generated in the water. For example, temperatures as high as 65°C were observed in wastewater effluent, following an ultrasound dose of 2.5×10^{5} $J.L^{-1}$ (Madge and Jensen, 2002). The use of ultrasound in conjunction with another, conventional disinfectant holds more promise. Indeed, the action of ultrasound in disintegrating suspended solids and damaging microorganisms can improve the efficacy of disinfection when used in conjunction with chlorine or UV light (Phull *et al.*, 1997; Neis and Blume, 2003; Oyane *et al.*, 2005). For instance, a combination of a relatively low dose of ultrasound, 1.7×10^{3} $J.L^{-1}$, followed by UV disinfection provided 3.1 $\log_{10}$ inactivation of *E. coli*, compared to just 1.7 $\log_{10}$ inactivation with UV alone (Neis and Blume, 2003).

Table 2.5. *Disinfection of grey water.*

Water	Prior treatment	Disinfection	Dose	Microorganism	log_{10} removal	Concentration in final effluent ($.100mL^{-1}$)	Reference
Grey water (synthetic)	None	Multi-media filtration	n/a	*E. coli* *Staph. aureus* *P. aeruginosa*	4.6 1.7 0.0	1.3 63 50119	Gross *et al.* (2007)
Grey water (synthetic)	Screening filter, aerobic bioreactor (Pilot scale)	Media filtration	n/a	Faecal coliforms	3.8	2	Surendran and Wheatley (1998)
Grey water (bathroom)	Screening filter, aerobic bioreactor (Full scale)	Media filtration	n/a	Total coliforms Faecal coliforms	3.6 1.8	995 4	
Grey water (bathroom)	Screening filter (1mm)	Sand filtration	n/a	Faecal coliforms	0.4	1.3×10^{5}	Friedler *et al.* (2006)
		Sand filtration + Chlorine	1.4 $mg.L^{-1}$ chlorine residual (30 min)	Faecal coliforms	5.5	0.0	
Grey water (all streams, including urine)	Reed bed	Sand filtration	n/a	Faecal coliforms	4.0	0.5	Fittschen and Niemczynowicz (1997)
Grey water (all streams)	None	Reed beds (rock or plastic media)	n/a	Faecal coliforms	0.5 – 4.8	$1500 – 1.1\times10^{6}$	Dallas and Ho (2005)
Grey water (synthetic)	None	Membrane filtration (MBR)	n/a	Total coliforms	7.0 (max.)	0 - 50	Jefferson *et al.* (2000)
Grey water (shower)	Screening filter (1mm)	Membrane filtration (MBR)	n/a	Faecal coliforms	2.3	68	Merz *et al.* (2007)
Grey water (bathroom)	Screening filter (1mm)	Membrane filtration (MBR)	n/a	Faecal coliforms	4.1	27	Friedler *et al.* (2006)
		Membrane filtration (MBR) + Chlorine	1.4 $mg.L^{-1}$ chlorine residual (30 min)	Faecal coliforms	5.5	0.0	

Table 2.5. continued. *Disinfection of grey water.*

Water	Prior treatment	Disinfection	Dose	Microorganism	log_{10} removal	Concentration in final effluent ($.100mL^{-1}$)	Reference
Grey water (shower, wash basin)	Screening filter	Membrane filtration (MBR)	n/a	Total coliforms	5.7	6.3	Grant *et al.* (2006)
			n/a	*E. coli*	4.4	<0.1	
			n/a	Somatic coliphage	3.5	2.9	
			n/a	fRNA coliphage	1.1	0.0	
		Membrane filtration (MBR) + UV	NR	Total coliforms	6.5	0.4	
Grey water (shower, bath)	Fluidised-bed reactor	UV	25-40 $mJ.cm^{-2}$	Total coliforms	NR	2 – 500	Nolde (1999)
				Faecal coliforms	NR	2 – 30	
Grey water (bathroom)	Screening filter (1mm), rotating biological contactor, sedimentation, sand filter	Chlorine	1 $mg.L^{-1}$ chlorine residual (30 min)	Faecal coliforms	4.7	0.1	Friedler *et al.* (2005)
Grey water (bath, wash basin)	Screening filter (0.3mm), sedimentation	Chlorine	75 $mg.L^{-1}$ initial chlorine (> 1 $mg.L^{-1}$ chlorine residual)		NR	0.0	March *et al.* (2004)

Data are mean, median, or ranges of values.
n/a = not applicable
NR = not reported
max. = maximum

2.3.2.3. Media filtration

The application of filtration for disinfection involves the physical separation of pathogenic microorganisms from water by passing the water through a porous medium that retains the microorganisms. Media filtration can take the form of basic sand filters, multi-media filtration systems, or reed beds. The removal of microorganisms by media filtration relies on their deposition within the media bed by either adsorption to the media surface or through entrapment in pores between media granules. Counteracting this deposition, however, is a process of microorganism dislodgement caused by movement of the applied water through the media bed. Dislodged microorganisms are transported by the water and may be deposited further down the media bed or ejected with the filtered effluent. Microorganisms deposited within biologically active media filters are subsequently inactivated by nutrient limitation, exposure to inhibitory secretions from competing bacteria (Stevik *et al.*, 2004), bacteriophage parasitism (Fischer *et al.*, 2006), and predation by protozoa (Decamp and Warren, 1998). Media filtration is typically employed for tertiary treatment of wastewater to remove improve water quality prior to further disinfection and to remove specific pathogenic microorganisms of concern, such as *Cryptosporidium* (Tufenkji *et al.*, 2006).

A study of 73 samples of filtered secondary wastewater effluent by Kay *et al.* (2007), revealed an average 1.4 $\log_{10}$ reduction in total coliforms. In a wastewater reclamation plant, dual media filtration with coagulation gave $\log_{10}$ removals of 0.4, 0.5, 1.0, and 1.5, for total coliforms, enterovirus, *Cryptosporidium* oocysts, and *Giardia* cysts, respectively (Rose *et al.*, 1996), indicating the effective removal of larger, protozoan pathogens by media filtration. Numerous factors impact microorganism retention through granular media filters, however, including: media type, hydraulic loading rate, influent loading regime, gas saturation, and influent characteristics. Many of these factors can be controlled to optimise filtration performance. A greater media surface area and lower hydraulic loading rate improves the retention of microorganisms due to an increased availability of adsorption sites and a longer contact time facilitating adsorption (Stevik *et al.*, 1999a, Stevik *et al.*, 1999b). For example, Stevik *et al.* (1999b) investigated *E. coli* removal through different media types and reported up to a

3.8 $\log_{10}$ reduction in *E. coli* when the influent loading rate was halved, and removal was linked to the specific surface area of the media. Constant influent loading of granular media filters is considered most effective for the retention of microorganisms because intermittent loading can encourage the remobilisation and transport of microorganisms deposited within the media (Powelson and Mills, 2001; Auset *et al.*, 2005). Increasing gas saturation within granular media has been shown to improve the retention of bacteria and viruses (Lance and Gerba, 1984; Wan *et al.*, 1994) due to the enhanced microbial adsorption to media in the presence of gas (Rijnaarts *et al.*, 1993; Wan *et al.*, 1994). Indeed, in the study by Wan *et al.* (1994), the retention rate of bacteria was proportional to the gas saturation of the porous media, with bacteria retention increasing from 8% in fully water-saturated conditions to 29% with air present.

Disinfection by media filtration is best for water sources low in organic matter, which can reduce microorganism retention by competing with microorganisms for adsorption sites or by modifying the microorganism surface charge and increasing repulsion forces with the media (Harvey *et al.*, 1989; Johnson and Logan, 1996; Powelson and Mills, 1998; Schijven and Hassanizadeh, 2000). Microorganisms attached to particles or microbial aggregates are likely to be more effectively removed than individual microorganisms due to their larger size increasing their propensity to be strained within the media (Yao *et al.*, 1971). Yao *et al.* (1971) showed that the removal of latex particles through porous media was dependent on size, with around 40% removal efficiency of 1.1 µm particles (approx. size of single *E. coli* bacterium) increasing to approx. 95% removal efficiency of larger 8 µm particles.

Reported microorganism removal from grey water by media filtration varies greatly. Friedler *et al.* (2006) reported a low 0.4 $\log_{10}$ removal of faecal coliforms in grey water by sand filtration, while Dallas and Ho (2005) observed 0.5 to 4.8 $\log_{10}$ removal of faecal coliforms through reed beds with rock or plastic media under varying conditions. More recently, Gross *et al.* (2007) reported a 4.6 $\log_{10}$ removal of *E. coli* in synthetic grey water passed through a multimedia filter containing crushed rock, plastic media, and peat. In the same system, a lower $\log_{10}$ removal of 1.7 for *Staph. aureus* was

observed and *P. aeruginosa* bacteria were not removed, indicating that removal efficiencies can differ between microorganisms. Importantly, the use of media filtration for disinfection does not provide a robust barrier to microorganisms and typically leaves microorganisms of concern in the effluent water (Table 2.5.), which may be acceptable for certain reuse applications but would require additional disinfection for others.

2.3.2.4. Membrane filtration

Membrane filtration involves the passing of water through a microfiltration (~0.1 – 0.9 µm pore size) or ultrafiltration (~0.01 – 0.09 µm pore size) membrane, under pressure. Microorganism retention is achieved by size exclusion, when the membrane pore size is smaller than the microorganism size. A significant level of retention can also be achieved with pore sizes larger than the targeted microorganism due to adsorption to the membrane, which is controlled by hydrophobic and electrostatic interactions (Madaeni, 1995; Madaeni, 1999; Van Voorthuizen *et al.*, 2001).

Membrane filtration is often used in conjunction with an activated sludge process as part of a membrane bioreactor (MBR). The combined process has been shown to provide superior removal of biodegradable organics when treating grey water, compared to membrane filtration alone (Jefferson et el., 2004; Ramon *et al.*, 2004; Friedler *et al.*, 2006). MBR systems are increasingly used for wastewater treatment in areas requiring high quality effluent and have been identified as well-suited to treatment and disinfection for water reuse (Jefferson *et al.*, 2000; Gander *et al.*, 2000; Zhang and Farahbakhsh, 2007).

Membrane filtration is typically most effective for the removal of larger pathogens, such as protozoa, followed by bacteria, and then viruses, due to size exclusion. To illustrate, a microfiltration MBR treating wastewater gave complete removal of both *Giardia* and *Cryptosporidium*, 4.5 – 5.0 $\log_{10}$ removal of the bacterial indicators Enterococci and *E. coli*, and 3.1 – 3.8 $\log_{10}$ removal of bacteriophages (Ottoson *et al.*, 2006). Similarly, Zhang and Farahbakhsh (2007) reported 5.6 $\log_{10}$ removal of total coliforms, and 4.1 – 4.3 $\log_{10}$ removal of bacteriophages for an ultrafiltration MBR treating wastewater.

Membrane filtration is generally more robust and less susceptible to microorganism breakthrough than media filtration, particularly when treating poorer quality effluents. However, bacteria have been shown to pass through membranes with nominal pore sizes smaller than the bacteria itself (Pall *et al.*, 1980). This can be attributed to the distribution of pore size in membranes, meaning that pores significantly larger than the nominal size are common in membranes (Sadr Ghayeni *et al.*, 1999; Phattaranawik *et al.*, 2003). The breakthrough of bacteria and viruses raises concerns about the disinfecting potential of membranes and the potential for bacterial regrowth. Indeed, bacteria that pass through the membrane may persist in a viable but non-culturable state (Sadr Ghayeni *et al.*, 1999).

Grant *et al.* (2006) investigated a microfiltration MBR (0.4 µm nominal pore size) system treating grey water and reported maximum $\log_{10}$ removals of 7.0 for total coliforms, 5.0 for *E. coli*, and 4.3 for somatic coliphages. In an ultrafiltration MBR treating grey water a 4.1 mean $\log_{10}$ removal of faecal coliforms was observed (Friedler *et al.*, 2006). MBR processes provide high $\log_{10}$ removals of microorganisms but low concentrations of coliform bacteria and bacteriophage are frequently detected in MBR-treated grey water effluent (Jefferson *et al.*, 2000; Friedler *et al.*, 2006; Grant *et al.*, 2006; Merz *et al.*, 2007). The regrowth of heterotrophic bacteria and faecal coliforms, by 0.8 and 0.3 $\log_{10}$ units, respectively, has also been observed in MBR-treated grey water effluent after seven days of storage (Friedler *et al.*, 2006). The application of a supplementary residual disinfectant must therefore be considered for MBR treated urban water destined for reuse.

2.3.3. Comparison of disinfection processes

2.3.3.1. Pathogen elimination

Disinfection processes vary in their efficacy of inactivation/removal for different types of pathogens. Chlorine, for example, is most effective for the inactivation of bacteria and viruses, and least for protozoa or spore-forming bacteria. UV light, in contrast, provides superior inactivation of certain pathogenic protozoa compared to bacteria or

viruses. Whereas disinfection by filtration tends to be size-related, with larger protozoan pathogens removed preferentially over bacterial, and then viral pathogens. Combining disinfection processes may be necessary to achieve pathogen-free water from urban waters containing a range of different pathogen types. Initial disinfection by media or membrane filtration followed by chlorine disinfection would prove a suitable combination, for example. Filtration processes provide efficient removal of protozoan pathogens while subsequent chlorine disinfection would grant effective inactivation of bacterial and viral pathogens, as well as leaving a residual disinfectant. Of the disinfectants reviewed, only chlorine and essential oils are capable of leaving a residual disinfectant in the water. The potential for regrowth of pathogenic microorganisms would be an important consideration for other disinfected waters.

2.3.3.2. Organic and particle sensitivity

Most disinfection processes are sensitive to the presence of organic or particulate material in water. Organic material reacts with chemical disinfectants, and absorbs UV light, reducing the available disinfectant for disinfection. This effect of organic material can be simply overcome by increasing the applied disinfectant dose to surpass the effect of the organic material. Organic material also reduces the disinfection capacity of media filtration systems. In contrast, the disinfection efficacy of membrane filtration and ultrasound technologies remain largely unaffected by organic material in water. Chemical and UV light disinfection is also sensitive to the presence of particulate material with embedded microorganisms, although this has not been demonstrated for essential oils. Intra-particle diffusion of chlorine and ozone to inactivate particle-associated pathogens is possible at elevated concentrations, although particle characteristics including size are limiting factors (Dietrich *et al.*, 2003; Dietrich *et al.*, 2007). In contrast, pathogens embedded in particulate material with no light pathway are completely shielded from inactivation by UV light, regardless of the applied dose. Particulate material has no impact on disinfection by ultrasound or membrane filtration and may actually improve microorganism removal by media filtration.

2.3.3.3. Application for urban reuse

Significant levels of organic and particulate material in both black water and grey water suggest that a form of pre-treatment would be required to permit effective disinfection. In grey water, this can range from coarse filtration to biological treatment, allowing subsequent disinfection with chlorine, UV light, or membrane filtration (Table 2.5.). Rain water is typically much less polluted than grey or black water in terms of organics, although particulate material is of concern, suggesting that media filtration may be particularly suitable for the disinfection of rain water. Ultimately, technology selection for the disinfection of urban water destined for reuse will depend on the desired water quality, which in turn, is dependent on the reuse application and the existence of regional standards or guidelines for urban water reuse.

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CHAPTER 3:
A STUDY OF THE MICROBIAL QUALITY OF GREY WATER AND AN EVALUATION OF TREATMENT TECHNOLOGIES FOR REUSE

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3. A STUDY OF THE MICROBIAL QUALITY OF GREY WATER AND AN EVALUATION OF TREATMENT TECHNOLOGIES FOR REUSE

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ABSTRACT

The reuse of grey water for non-potable water applications is a potential solution for water-deprived regions worldwide. Adequate treatment of grey water prior to reuse is important to reduce the risks of pathogen transmission and to improve the efficacy of subsequent disinfection. This study investigated the presence of common pathogens in grey water and compared the pathogen removal performance of leading contender treatment technologies. The opportunistic pathogens *Pseudomonas aeruginosa* and *Staphylococcus aureus* were detected in the grey water tested. Three configurations of constructed wetland, a membrane bioreactor (MBR), and a membrane chemical reactor (MCR) were evaluated for indicator bacteria (total coliforms, *Escherichia coli*, Enterococci, Clostridia, and heterotrophs) removal over a period of two years under conditions of low and high strength grey water influent. Total coliforms were found to be good indicators for *P. aeruginosa*, showing strong and significant Spearman's rank correlations in the influent grey water (r_s = 0.77, P = 0.005) and treated effluents (r_s = 0.81, P = <0.001). The MBR provided the highest quality treated effluent and was the most robust treatment technology, remaining unaffected by an increase in influent grey water strength. Of the three constructed wetlands, the VFRB was the most reliable performer under low and high strength influent conditons, indicating aerobic unsaturated wetland to be the most suitable form of the technology for pathogen removal.

3.1. INTRODUCTION

The reuse of grey water for non-potable applications, such as toilet flushing or irrigation, can substantially reduce potable water consumption (FBR, 2005). Treatment and disinfection of grey water are important to provide water that is both safe and aesthetically appropriate for reuse. Inadequately treated or disinfected grey water presents a risk of infection to end users from pathogens in the reused grey water. To date, no incidences of illness linked to grey water reuse have been reported and so the health risks appear to be low, however, studies on the health impacts of grey water reuse are limited. The pollutant load of grey water is less than that of domestic sewage but the occurrence of faecal indicator bacteria in grey water (Casanova *et al.* 2001a; Ottoson and Stenström, 2003a; Friedler, 2004), demonstrates the potential presence of faecally-transmitted pathogens. There is limited data in the literature on specific pathogens in grey water, however, the opportunistic pathogen *Pseudomonas aeruginosa*, and the protozoa, *Cryptosporidium* and *Giardia*, have been detected in grey water (Casanova *et al.*, 2001b; Birks *et al.*, 2004).

Grey water treatment technologies must be robust to handle variations in organic and pathogen concentration in grey water influent, and to consistently produce effluent of an appropriate and safe quality to meet required standards for reuse. Microbiological standards for urban reuse vary worldwide (Table 3.1.), from the stringent guidelines of the USEPA and the State of California, to the less demanding Germany guidelines (based on EU standards for recreational waters), to a lack of national standards in the UK. Biological processes, which range from state-of-the-art membrane bioreactors to low-tech constructed wetland systems, are considered most appropriate for treatment of grey water because of their efficient removal of organics (Jefferson *et al.*, 2001; Pidou *et al.*, 2007). The quality of treated effluent is important because of its impact on reuse applications (Wiel-Shafran *et al.*, 2006) and downstream disinfection. Organics increase disinfectant demand in treated effluent, reducing the efficacy of disinfection (LeChevallier *et al.*, 1981), and provide substrate for pathogen regrowth (Narkis *et al.*, 1995). The removal of suspended solids is also important as particulate matter can shield pathogens from disinfection (Dietrich *et al.*, 2003).

Table 3.1. *Water quality standards/guidelines for urban wastewater reuse.*

Urban water reuse standards/guidelines	Water quality	Microbiological (CFU.100mL^{-1})
USEPA (2004)	BOD_5 ≤10 mg.L^{-1} Turbidity ≤ 2 NTU pH = 6-9	Faecal coliforms = ND Viable pathogens = ND
USA, California (USEPA 2004)	Turbidity = 2 NTU Avg (5 NTU Max)	Total coliforms = 2.2 Avg (23 Max in 30 days)
Germany (Nolde, 1999)	BOD_7 <5 mg.L^{-1}	Total coliforms <10000 Faecal coliforms <1000 *Pseudomonas aeruginosa* <100

Avg: average, Max: maximum, ND: none detectable

This study investigated the presence of specific pathogens and indicator bacteria in grey water and evaluated the performance of leading contender treatment technologies for grey water reuse.

3.2. MATERIALS AND METHODS

3.2.1. Grey water

Grey water was collected from bathroom sinks, baths and showers of 18 specially plumbed student flats on the Cranfield University campus. The grey water was held in a continuously circulating primary holding tank, prior to distribution to the treatment technologies. To enable different feed strengths to be tested a supplementary dosing system was installed. The high strength supplementary solution was a 10% v/v mixture of Tesco Value shampoo in tap water. The high strength supplementary solution and the real grey water were pumped, at a ratio of 1:55, into a second holding tank, from which the mixture was pumped to the treatment technologies. The real grey water is referred to in this paper as low strength and the supplemented grey water as high strength. The treatment technologies were fed low strength grey water from July 2004 to September 2005, and high strength grey water from October 2005 to August 2006.

3.2.2. Grey water treatment technologies

Grey water was fed to three constructed wetland systems, a membrane bioreactor (MBR), and a membrane chemical reactor (MCR) (Table 3.2.). The constructed wetlands, established during June 2004, included two reed beds (RIBs, Oceans-ESU, Bradford, UK). One reed bed was operated in horizontal flow (HFRB, also referred to as subsurface flow) and the other in vertical flow configuration (VFRB). The third constructed wetland (Green Roof Water Recycling System – GROW, Water Works UK, London) comprised a system of shallow troughs filled with clay and gravel media and a variety of aquatic plants. Aeration was provided through the flow of water down weirs at the end of each row and via a porous pipe at the base of the troughs through which air was bubbled for one hour each day. The constructed wetlands were fed grey water at a rate of 480L.d^{-1}. The GROW and HFRB were fed continuously via peristaltic pumps at a constant flow rate, while the VFRB was batch fed via a network of pipes across the reed bed surface. The MBR was operated in continuous flow. Aeration under the membranes provided scouring and maintained aerobic conditions while a recirculation loop provided mixing of the biomass.. The MCR combined an advanced oxidation process with membrane filtration. A photo-catalytic reaction was created by combining ultraviolet (UV) light with titanium dioxide in a closed reactor. The treated MBR and MCR effluents were extracted by peristaltic pump.

3.2.3. Analysis of grey water and treated effluents

For testing of *Staphylococcus aureus, P. aeruginosa, Salmonella* spp. and *Campylobacter* spp., unsupplemented grey water samples were collected from the holding tank in sterile polypropylene bottles. A maximum grey water volume of 1L was analysed for each pathogen. In addition, cumulative swab samples were taken for testing of *Salmonella* and *Campylobacter*. The cumulative swab samples were taken using a sterile cotton swab, which was suspended in the unmodified, low strength grey water holding tank for seven days. Detection/enumeration and confirmatory tesing of *Staph. aureus, P. aeruginosa, Salmonella* spp. and *Campylobacter* spp. were performed in

Table 3.2. *Pilot scale grey water treatment technologies.*

Treatment technology	Operating conditions
Horizontal flow reed bed (HFRB)	$6m^2$ surface area, 0.7m depth Sand/soil/compost mix media (≤1mm diameter) Planted with *Phragmites australis* HLR: $480L.d^{-1}$ continuous flow HRT: 2.1d
Vertical flow reed bed (VFRB)	$6m^2$ surface area, 0.7m depth Sand/soil/compost mix media (≤1mm diameter) Planted with *Phragmites australis* HLR: $480L.d^{-1}$ in 10 batches of 48L HRT: 2h per batch
Green roof water recycling system (GROW)	Five rows of shallow troughs ($1.2m^2$ x 0.1m depth) Optiroc expanded clay media (~10mm diameter) topped with gravel chippings (~20mm diameter) Planted with a variety of aquatic plants Baffles and weirs create plug flow Additional aeration for 1hr per day HLR: $480L.d^{-1}$ continuous flow HRT: 2.1d
Membrane bioreactor (MBR)	Two joint 34L reactors, each fitted with two submerged A4 flat sheet Kubota membranes, 0.4 µm nominal pore size Seeded with activated sludge biomass Aeration: 5 $L.min^{-1}$ Recirculation loop generated by air lift (10 $L.min^{-1}$) Flux: 15 $L.m^{-2}.h^{-1}$ HLR: $168L.d^{-1}$ HRT: 9.7h Solids retention time: 68d
Membrane chemical reactor (MCR)	9L reactor with four submerged 25W UV-C lamps and side air-lift tubular membrane, 0.05 µm nominal pore size 5 $g.L^{-1}$ titanium dioxide Aeration: 5 $L.min^{-1}$ Recirculation loop generated by air lift (10 $L.min^{-1}$) Flux: 15 $L.m^{-2}.h^{-1}$ HLR: $57L.d^{-1}$ HRT: 3.8h

HLR: hydraulic loading rate
HRT: hydraulic retention time

duplicate and carried out in accordance with standard methods (Environment Agency, 2002; Health Protection Agency, 2003). Total coliforms, *Escherichia coli*, Enterococci, sulphite-reducing Clostridia, *P. aeruginosa*, and heterotrophic bacteria were routinely enumerated in grey water and treated effluents by standard methods (Environment

Agency 2002). Heterotrophic bacteria were enumerated on R2A agar (Oxoid) and incubated at 30°C for 3 days. To include data with a value of less than one, microbial data is reported as $\log_{10}(y+1)$, with standard deviation. The MBR and MCR effluents were not included in the microbiological analyses during the low strength grey water influent conditions. The Spearman rank correlation was used to test the relationships between indicator bacteria and *P. aeruginosa* in the influent grey water (low and high strength) and the treated effluents. Biological oxygen demand (BOD_5), chemical oxygen demand (COD), total organic carbon (TOC), total suspended solids (TSS), and turbidity were analysed routinely over the low and high strength testing periods by standard methods (APHA, 1998). COD and TOC were analysed using the Merck cell vial spectroquant method.

3.3. RESULTS

3.3.1. Chemical/Physical treatment performance

At low strength, all five treatment technologies provided BOD removal of 86% or greater, with effluent BOD concentrations of 3 $mg.L^{-1}$ or lower (Table 3.3.). COD removal was more varied, with the VFRB and GROW reducing COD to 21 and 19 $mg.L^{-1}$, respectively; the HFRB reducing the COD level to 29 $mg.L^{-1}$; while the MBR and MCR effluents had the highest COD values of 47 and 43 $mg.L^{-1}$, respectively. The VFRB and GROW also gave high suspended solids removal of 90% or more compared to the HFRB with 69% suspended solids removal. The GROW, MBR, and MCR effluents had the lowest turbidity values, at less than 1 NTU. The turbidity of the two reed bed effluents were considerably higher at 8.1 and 16.9 NTU for the VFRB and HFRB, respectively.

The high strength grey water had increased values for water quality parameters compared to the low strength grey water. Concentrations of BOD and COD, for example, were 164 and 495 $mg.L^{-1}$, respectively, at high strength, compared to 20 and 87 $mg.L^{-1}$, respectively, at low strength (Table 3.3.). BOD removals by the HFRB and

GROW were reduced under high strength influent conditions to 75% and 68%, respectively. In contrast, the VFRB and MBR maintained high BOD removals of 97% and 99%, respectively. Similarly, the VFRB and MBR effluents showed only small increases in COD concentration of 10 and 6 $mg.L^{-1}$, respectively, compared to the HFRB and GROW effluents, which had COD values 95 and 140 $mg.L^{-1}$ higher than when treating the low strength grey water. TSS levels in the constructed wetland effluents increased but were low in the MBR and MCR, at <2 $mg.L^{-1}$. The turbidity of the HFRB and VFRB effluents was reduced under high strength conditions, most significantly in the VFRB, to 2.2 NTU. In contrast, the turbidity of the GROW increased from 0.8 NTU at low strength to 28.8 NTU at high strength.

Table 3.3. *Chemical/physical properties of low and high strength grey water influents, and treated effluents.*

Parameter		Grey water	HFRB	VFRB	GROW	MBR	MCR
Low strength							
BOD	Mean	20	2	1	2	1	3
($mg.L^{-1}$)	STD	11	1	1	1	1	2
	n	80	79	81	80	40	7
COD	Mean	87	29	21	19	47	43
($mg.L^{-1}$)	STD	38	9	6	8	13	14
	n	51	51	51	50	40	7
TSS	Mean	29	9	2	3	ND	ND
($mg.L^{-1}$)	STD	32	8	2	3	ND	ND
	n	82	82	82	80	ND	ND
Turbidity	Mean	19.6	16.9	8.1	0.8	0.2	0.1
(NTU)	STD	14	16	10	2	0.1	0.0
	n	84	83	84	83	40	7
High strength							
BOD	Mean	164	57	5	80	1	10
($mg.L^{-1}$)	STD	39	32	6	38	2	8
	n	23	23	23	23	57	16
COD	Mean	495	124	31	159	53	78
($mg.L^{-1}$)	STD	192	50	30	64	24	18
	n	12	12	12	12	57	16
TSS	Mean	93	34	10	20	1	2
($mg.L^{-1}$)	STD	66	15	6	8	2	1
	n	24	24	24	24	20	5
Turbidity	Mean	67.4	12.3	2.2	28.8	0.2	0.7
(NTU)	STD	92.3	13.4	1.5	10.7	0.1	1.1
	n	25	25	25	25	57	16

Data shown are mean values with standard deviation (STD) and sample number (n). ND = no data.

The MBR turbidity was unchanged at 0.2 NTU. The MCR effluent showed increases in BOD, COD, and turbidity when receiving high strength compared to low strength influent. The mean pH of the treated effluents were between 6.8 and 7.3, and mean ammonia concentrations were below 1 $mg.L^{-1}$, for both low and high strength treated effluents.

3.3.2. Microbial quality of grey water

Attempts to identify specific pathogens in grey water revealed that *P. aeruginosa* was present in all the grey water samples tested, at a mean concentration of 4.4±0.6 $log_{10}CFU.100mL^{-1}$ (Table 3.4.). Whereas, *Staph. aureus* was identified in the grey water in 25% of the samples tested, at a mean concentration of 3.4 $log_{10}CFU.100mL^{-1}$. Presumptive *Salmonella* spp. and *Campylobacter* spp. were identified from the cumulative swab and 1000mL filtered samples but proved negative following confirmatory testing. Total coliform, *E. coli*, Enterococci, and Clostridia concentrations in the low strength grey water were 5.4±0.8, 2.8±0.8, 2.8±0.9, and 3.1±0.7 $log_{10}CFU.100mL^{-1}$, respectively (Figure 3.1.). In the high strength grey water, the concentration of total coliforms and *P. aeruginosa* were approximately 2 log_{10} units greater compared to the low strength grey water, at 7.3±0.5 and 6.8±0.2 $log_{10}CFU.100mL^{-1}$, respectively (Figure 3.2.). The concentration of *E. coli* was almost 1 log_{10} unit greater, while mean numbers of Enterococci and Clostridia remained similar to the low strength grey water. Heterotrophic bacteria in the grey water increased from 7.0±0.5 $log_{10}CFU.mL^{-1}$ at low strength to 7.8±0.2 $log_{10}CFU.mL^{-1}$ at high strength (Figure 3.3.).

3.3.3. Microbiological treatment performance

Of the three constructed wetlands treating the low strength grey water, the HFRB gave the poorest removal of indicator bacteria, and the VFRB provided the best overall removal. To illustrate, the HFRB gave 2.8 log_{10} removal of total coliforms, compared to a 4.7 log_{10} removal by the VFRB (Figure 3.1.).

Table 3.4. *Pathogens in grey water (low strength).*

Microorganism	No. samples tested	% samples with presumptive microorganisms	% samples with confirmed microorganisms	Mean concentration of confirmed microorganisms ± standard error ($log_{10}CFU.100mL^{-1}$)
Pseudomonas aeruginosa	9	100	100	4.43 ± 0.19
Staphylococcus aureus	8	100	25	3.37 ± 0.12
Salmonella spp.	13	54	0	n/a
Campylobacter spp.	9	67	0	n/a

Under the high strength grey water influent conditions, the HFRB provided similar mean log_{10} removals as when treating low strength grey water influent, with a log_{10} removal of 3.0 for total coliforms, for example (Figure 3.2.). The VFRB demonstrated a reduced log_{10} removal of 3.1 for total coliforms under high strength conditons. The treatment performance of GROW was most greatly affected by the change from low to high strength influent with reduced log_{10} removals for all bacteria tested. To illustrate, log_{10} removal of total coliforms was 1.5, compared to 3.7 under low strength conditions. The MBR effluent was negative for total coliforms in 33% of samples and low numbers were observed overall, with a mean concentration of 0.6 $log_{10}CFU.100mL^{-1}$. The MCR effluent did not contain total coliforms in the two samples tested.

The three specific faecal indicator bacteria, *E. coli*, Enterococci, and Clostridia, were present in similar numbers in the low strength grey water: 2.8, 2.8, and 3.1 $log_{10}CFU.100mL^{-1}$, respectively. Despite similar initial concentrations, there were clear differences in their removal by the constructed wetland treatment technologies. *E. coli* were most effectively removed, followed by Enterococci, and then Clostridia. To illustrate, the VFRB gave log_{10} removals of 2.8, 2.3, and 2.0 for *E. coli*, Enterococci, and Clostridia, respectively. A similar trend of removal was observed under high strength conditions. Numbers of heterotrophic bacteria were increased in all three constructed wetland effluents under high strength conditions. In the VFRB effluent, for example, the mean concentration of heterotrophs was 3.7±0.3 when receiving low strength influent, and 5.6±0.9 $log_{10}CFU.ml^{-1}$ when treating the high strength grey water

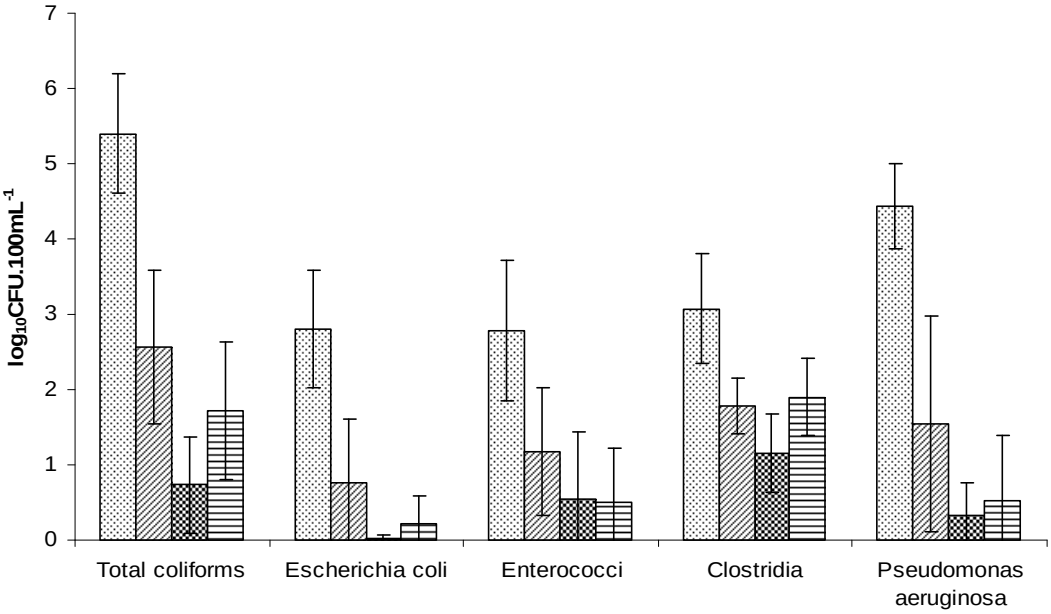

Figure 3.1. *Indicator bacteria and P. aeruginosa in low strength grey water and treated effluents from July 2004 to September 2005. Influent grey water (dots), HFRB effluent (diagonal lines), VFRB effluent (squares), and GROW effluent (horizontal lines). Data are mean values with standard deviation, n = 47-57, 50-58, 49-56, 11-12, and 8-9 for total coliforms, E. coli, Enterococci, Clostridia, and P. aeruginosa, respectively.*

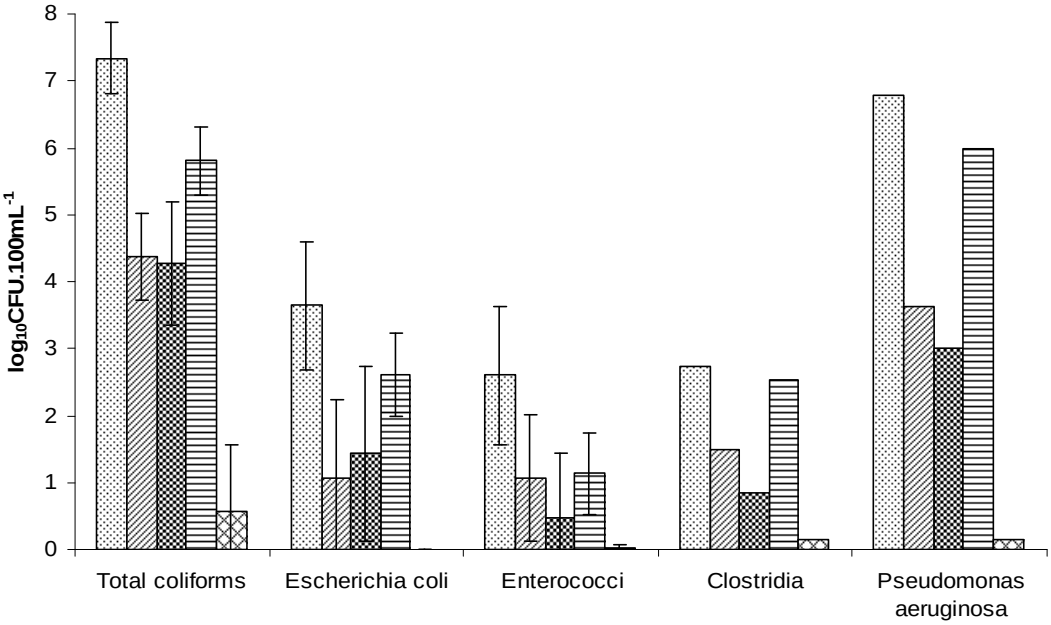

Figure 3.2. *Indicator bacteria and P. aeruginosa in high strength grey water and treated effluents from October 2005 to July 2006. Influent grey water (dots), HFRB effluent (diagonal lines), VFRB effluent (squares), GROW effluent (horizontal lines), and MBR effluent (crosses). Data are mean values with standard deviation, n = 16-21, 19-21, 19-21, 2, and 2 for total coliforms, E. coli, Enterococci, Clostridia, and P. aeruginosa, respectively.*

influent (Figure 3.3.). The concentration of heterotrophic bacteria in the MCR effluent was 5.4±0.2 $log_{10}CFU.mL^{-1}$, similar to that of the HFRB and VFRB, while the concentration in the MBR effluent was lowest, at 4.4±0.7 $log_{10}CFU.mL^{-1}$.

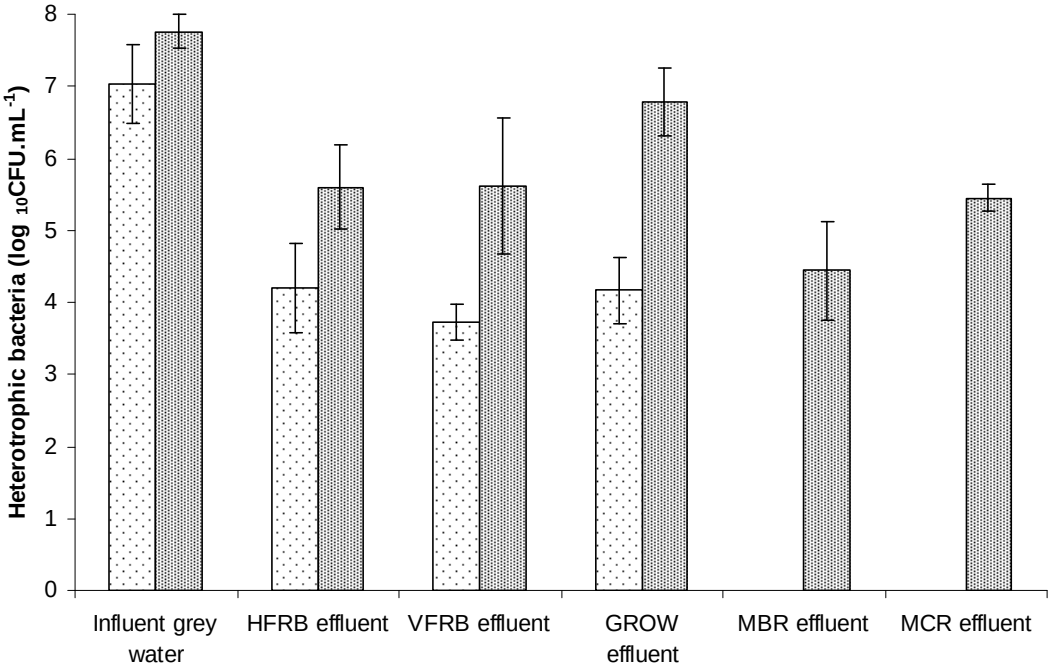

Figure 3.3. *Heterotrophic bacteria in low strength (lightly shaded) and high strength (heavily shaded) grey water and treated effluents. Data are mean values with standard deviation, n = 15-38 for low strength, and 17-18 for high strength conditions.*

The three constructed wetlands showed variation in their removal of indicator bacteria over the course of the study. Total coliform removal by the HFRB improved in the warmer summer months, coinciding with an increase in effluent temperature (Figure 4.4.). In July, when the effluent temperature reached a peak of 20°C, numbers of total coliforms in the HFRB effluent dropped to just 0.15 $log_{10}CFU.100mL^{-1}$, lower than the VFRB and GROW. The VFRB total coliform removal performance showed no obvious seasonal variation. However, a trend of steadily decreasing total coliform removal over time is evident, with total coliform numbers increasing from 0.1 to 2.4 $log_{10}CFU.100mL^{-1}$ when receiving low strength grey water, and from 3.3 to 5.7 $log_{10}CFU.100mL^{-1}$ over the period receiving high strength grey water influent. GROW appeared unaffected by changes in temperature or season with total coliform numbers remaining relatively consistent: between 1.0 and 1.4 $log_{10}CFU.100mL^{-1}$ when receiving

low strength influent, and between 5.4 and 6.6 $log_{10}CFU.100mL^{-1}$ while receiving high strength influent.

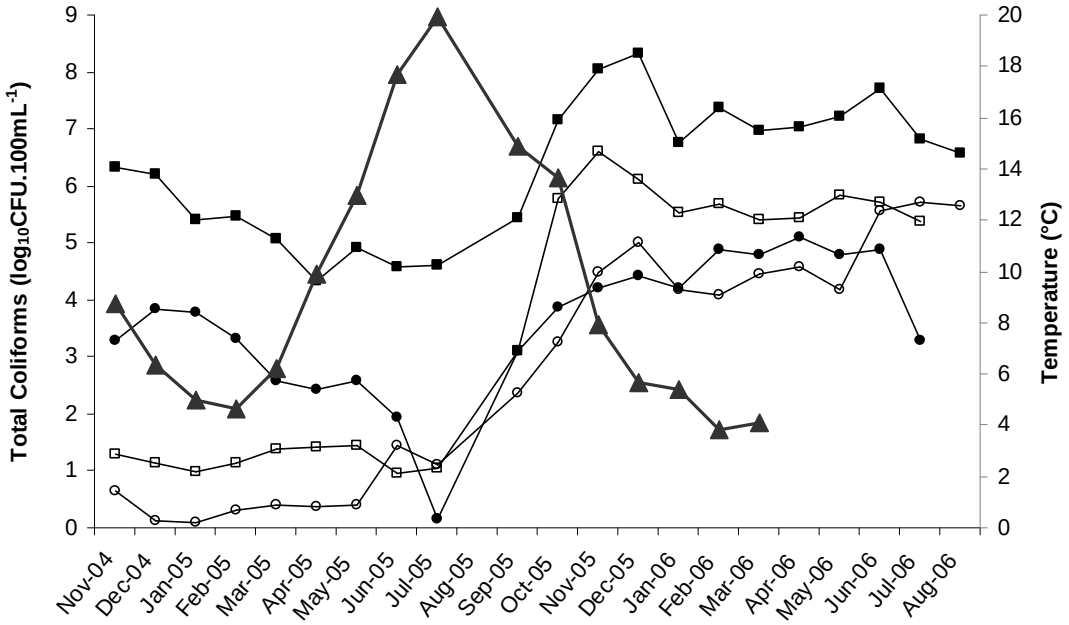

Figure 3.4. *Mean total coliforms in influent grey water (■), HFRB effluent (●), VFRB effluent (○), and GROW effluent (□); and mean effluent temperature (▲), under low strength (November 2004 to September 2005) and high strength (October 2005 to August 2006) influent grey water conditions, n = 2-6.*

The pattern of *P. aeruginosa* removal across the treatment technologies was closely matched to that of total coliform removal. Log_{10} removals of *P. aeruginosa* from low strength grey water, for example, were 2.9, 4.2, and 3.9; similar to those of total coliforms, 2.8, 4.7, and 3.7; for the HFRB, VFRB, and GROW, respectively. Statistical analysis of the relationships between indicator bacteria and *P. aeruginosa* (Table 3.5.), revealed strong, significant correlations between total coliforms and *P. aeruginosa* in the influent grey water ($r_s = 0.77$, $P = 0.005$) and in the treated effluents ($r_s = 0.81$, $P = <0.001$). Significant correlations were also observed between *E. coli* and *P. aeruginosa* but the correlations were not as strong as with total coliforms. No significant correlation between Enterococci or Clostridia, and *P. aeruginosa* was observed. There was a significant correlation between heterotrophic bacteria and *P. aeruginosa* in the treated effluents, however, the correlation was weak ($r_s = 0.50$, $P = 0.011$).

Considering the performance of the treatment technologies against established quality standards for reuse revealed that, under low strength grey water influent conditions, the three constructed wetlands comfortably met the Germany reuse standard for total coliforms and *P. aeruginosa* of less than 10000 and 100 CFU.100mL^{-1} (4.0 and 2.0 $\log_{10}$CFU.100mL^{-1}), respectively (Figure 3.5.). The more stringent California State total coliform standard of 2.2 per 100mL^{-1} (equivalent to 0.51 $\log_{10}$(y+1) CFU.100mL^{-1}) was not met by the HFRB or GROW effluent with any of the tested samples. The VFRB effluent met the total coliform standard in 44% of the effluent samples tested and was closest to meeting the standard with a mean total coliform concentration of 0.7±0.6 $\log_{10}$ CFU.100mL^{-1} for the testing period. All three constructed wetlands met the BOD requirements of the USEPA guidelines, while only the GROW was able to meet the turbidity requirement of less than 2 NTU. When treating the high strength grey water, none of the three constructed wetlands met the Germany reuse standard for total coliforms and *P. aeruginosa* (Figure 3.6.). The VFRB came the closest with mean concentrations of 4.3 and 3.0 $\log_{10}$CFU.100mL^{-1} for total coliforms and *P. aeruginosa*, respectively. The MBR effluent met the Title 22 total coliform standard in 74% of the samples tested and met the Germany standard in 100% of the samples tested. The MBR effluent also met the USEPA guidelines for BOD and turbidity. The VFRB was the only constructed wetland able to meet the USEPA guideline for BOD under the high strength conditions.

3.4. DISCUSSION

3.4.1. Chemical/Physical quality of grey water

The unmodified, low strength grey water used for this study was of low organic strength compared to literature data for grey water from similar sources. Friedler (2004) reported BOD values of between 173 and 424 mg.L^{-1}, COD values of between 230 and 645 mg.L^{-1} and total suspended solids values of between 78 and 303 mg.L^{-1} for grey water from the bath, shower, and wash basin. Meanwhile, Jefferson *et al.* (2004) have reported BOD values of between 129 and 155 mg.L^{-1}, and COD values of between 367 and 587 mg.L^{-1}, also for grey water from the bath, shower, and wash basin. These data

were for individual grey water streams sampled directly and, in the study of Jefferson *et al.* (2004), immediately analysed or stored below 5°C before analysis. The grey water used in this study was taken from a real grey water collection system and was therefore mixed, transported through pipework, and stored, before being distributed to treatment technologies or sampled for analysis. Microbial activity during this period of transport and storage may be responsible for the relatively low organic strength of the grey water and for the relatively high numbers of indicator bacteria observed. The supplemented 'high strength' grey water used in this study had greater BOD, COD and TSS values of 164, 495, and 93 $mg.L^{-1}$, respectively, comparable to the more polluted grey water sources reported in literature.

Table 3.5. *Spearman's rank correlation of indicator bacteria with P. aeruginosa in influent grey water (n=11) and treated effluents (n=32).*

Indicator bacteria	Influent grey water	Treated effluents
Total coliforms		
r_s	0.77	0.81
P	0.005	<0.001
E. coli		
r_s	0.69	0.65
P	0.019	0.001
Enterococci		
r_s	-0.19	0.41
P	0.573	0.131
Clostridia		
r_s	-0.16	0.17
P	0.650	0.419
Heterotrophs		
r_s	0.34	0.50
P	0.312	0.011

3.4.2. Microbial quality of grey water

The mean concentration of total coliforms in the low strength grey water, 5.4±0.8 $log_{10}CFU.100mL^{-1}$ (Figure 3.1.) is at the high end of literature data for grey water from similar sources (bathroom sink, shower and bath), of between 3.0 and 5.5 $log_{10}CFU.100mL^{-1}$ (Rose *et al.*, 1991; Nolde, 1999; Eriksson *et al.*, 2003; Jefferson *et al.*, 2004). A range of faecal indicator bacteria were consistently enumerated from the

unmodified, low strength grey water in this study (Figure 3.1.), similarly to previous studies (Rose *et al.*, 1991; Nolde, 1999; Ottoson and Stenström, 2003a; Friedler *et al.*, 2005). It is clear that faecal contamination of grey water is a common occurrence and therefore the potential exists for a range of faecally-transmitted pathogens to be passed into grey water. However, the enteric pathogens *Salmonella* spp. and *Campylobacter* spp. were not detected in the current study, supported by the failure of previous attempts to isolate these faecally-transmitted bacteria in grey water (Christova-Boal *et al.*, 1996; Birks *et al.*, 2004). However, faecally-transmitted protozoa, *Giardia* and *Cryptosporidia*, have been detected in hand basin grey water collected in a large facility (Birks *et al.*, 2004).

The opportunistic pathogen *P. aeruginosa* was consistently present in the tested grey water samples at a mean concentration of 4.4±0.6 $\log_{10}$CFU.100mL^{-1}. This is very similar to the findings of Casanova *et al.* (2001b) who reported the presence of *P. aeruginosa*, at a mean concentration of 4.3 $\log_{10}$CFU.100mL^{-1}, in all samples of domestic grey water taken from a single household. Casanova *et al.* (2001b) reported finding no *Staph. aureus*, however, which was recovered in grey water samples tested in this study. *P. aeruginosa* and *Staph. aureus* are opportunistic pathogens that cause respiratory and skin infections, particularly in susceptible individuals, such as the elderly, young, and immunocompromised. This is evidenced by their prevalence in hospital-aquired infectons (Anaissie *et al.*, 2002; Wertheim *et al.*, 2005). The presence of opportunistic pathogens in grey water indicates that inadequately treated and disinfected grey water may pose a particular risk to vulnerable individuals within households reusing grey water.

Data from this study showed the regrowth of bacteria in grey water, stimulated by an increase in organic concentration. Total coliforms, *E. coli* and *P. aeruginosa* increased in concentration by 1.9, 0.8, and 2.2 $\log_{10}$ units, respectively, with the addition of synthetic supplement to create high strength grey water. The consistent presence of *P. aeruginosa*, coupled with its ability to regrow in grey water, marks it as an opportunistic pathogen of concern for grey water reuse. The current results add to previous studies which have shown regrowth in terms of indicator microorganisms. For instance, Rose *et*

al. (1991) reported growth of coliform bacteria of between 1 and 2 log_{10} units in stored grey water after 48 hours. Further, Dixon *et al.* (1999) measured total coliforms in stored bath grey water and observed a total coliform concentration increase of over 2 log_{10} units, from 1.7 to >4.0 $log_{10}.100mL^{-1}$ within 24 hours. In contrast, previous studies of the enteric bacteria, *Salmonella* sp. and *Campylobacter* sp. seeded into grey water have indicated that no regrowth occurs (Rose *et al.*, 1991; Ottoson and Stenström, 2003b). It has been suggested that the lack of regrowth is due to the presence of competing microorganisms, leading to rapid die-off or entry into a viable but non-culturable state (Ottoson and Stenström, 2003b).

3.4.3. Microbiological treatment performance

Log_{10} removals of indicator bacteria by constructed wetlands in the current study are comparable to those reported previously for treatment of grey water (Dallas and Ho, 2005) and wastewater (Green et al, 1997; Hench *et al.*, 2003; Greenway, 2005). For instance, previous research has shown log_{10} faecal coliform removals of 4.5 to 4.6 for the treatment of grey water through horizontal flow reed beds with rock or plastic media (Dallas and Ho, 2005). Gross *et al.* (2007) reported a log_{10} *E. coli* removal of 4.6 through a vertical media filter treating synthetic grey water. In the current study, comparison between the three constructed wetland systems revealed differences in the overall levels of removal, and differences in their responses to both seasonal variation and the transition from low to high strength grey water influent.

Seasonal temperature changes strongly influenced removal in the HFRB (Figure 4.4.), whereby removal increased as the temperature increased, but did not affect performance of the VFRB or GROW. Previous studies on HFRBs treating wastewater support this observation (Axler *et al.*, 2001; Quiñónez-Díaz, 2001; Karathanasis *et al.*, 2003). The influence of seasonal temperature change indicates that the inactivation of microorganisms through the HFRB, as opposed to physical adsorption or filtration, was a key mechanism for removal. Inactivation of bacteria within constructed wetlands occurs through competition for nutrients and exposure to inhibitory secretions from other bacteria (Stevik *et al*, 2004), virus-induced lysis (Fischer *et al.*, 2006) and

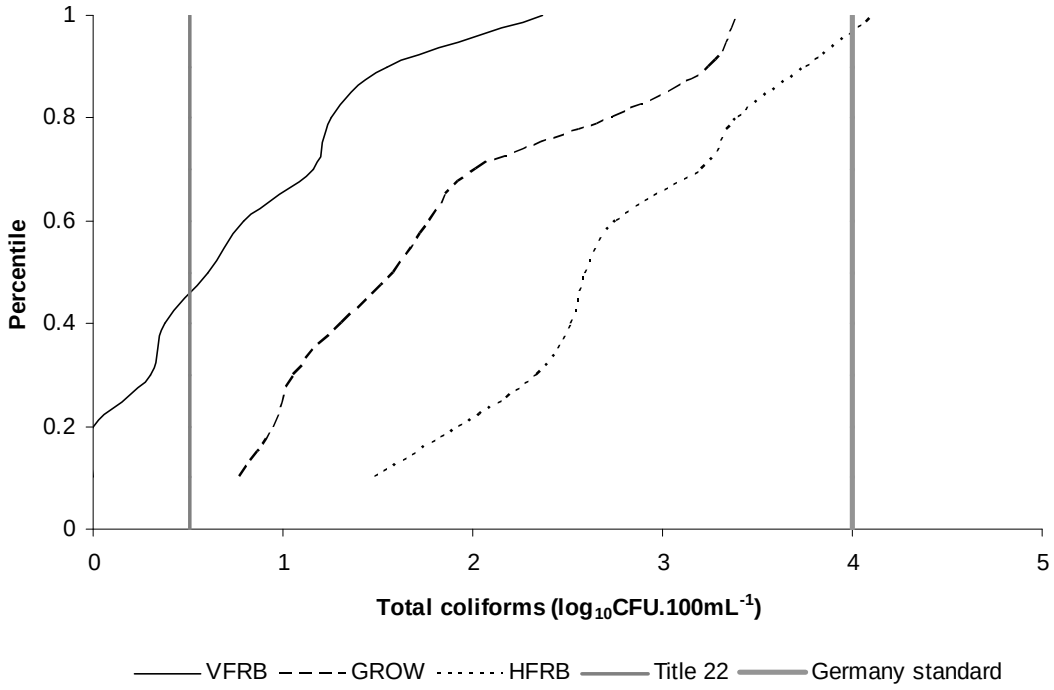

Figure 3.5. *Distribution of total coliform values for HFRB (n = 47), VFRB (n = 51), and GROW (n = 56) effluents treating low strength influent grey water. The California State Title 22 and Germany total coliform standards are included for reference.*

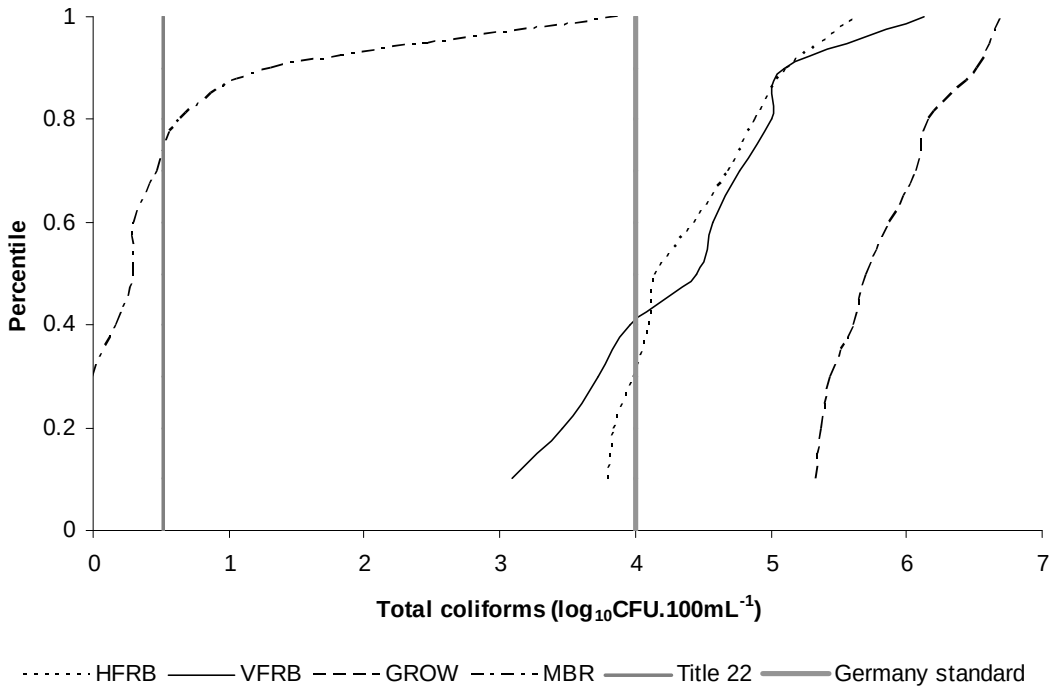

Figure 3.6. *Distribution of total coliform values for HFRB (n = 21), VFRB (n = 20), GROW (n = 20), and MBR (n = 16) effluents treating high strength influent grey water. The California State Title 22 and Germany total coliform standards are included for reference.*

predation by protozoa (Decamp and Warren, 1998). The fact that no change in overall removal levels was observed when comparing the low and high strength conditions suggests that competition for nutrients was not critical as nutrient availability would have been greater in the latter case.

Analysis of the data during the low strength phase revealed the VFRB to give superior removal to the other wetlands (Figure 3.1.). The only difference between the VFRB and HFRB in the current study was related to the influent loading regime. The VFRB was fed in batch drain cycles such that it was unsaturated (presence of gas) and effectively aerobic. Whereas the HFRB was fed continuously, was saturated and effectively anoxic. Microorganism removal in unsaturated media columns has been shown to be a balance between adsorption and dislodgement (Powelson and Mills, 2001) with the retention rate being proportional to the level of gas saturation (Wan *et al.*, 1994) due to the preference for bacteria to adhere to gas-water or gas-solid interfaces (Wan *et al.*, 1994; Rijnaarts *et al.*, 1993). Comparison to studies on clean sand indicate higher levels of removal in the VFRB, suggesting that the development of a biologically active media enhances attachment and/or reduces dislodgement during the 2 hour HRT. Reduced removal levels were observed under high strength conditions where the water was supplemented with an organic- and surfactant-rich shampoo. Organics and surfactants are known to reduce bacterial adsorption in porous media by competing for adsorption sites (Stevik *et al.*, 2004) and reducing the affinity of bacterial surfaces for adsorption (Powelson and Mills, 1998). Overall, the data shows that the VFRB achieved removal levels that were similar to or better than the HFRB under all conditions indicating that aerobic unsaturated removal pathways are more appropriate in cases of grey water treatment.

3.4.4. Implications for pathogen removal

The significant correlation between the opportunistic pathogen *P. aeruginosa* and total coliforms in the treated effluents, suggests that *P. aeruginosa* bacteria were removed or inactivated by similar processes as total coliform bacteria. The data shows that total coliforms were the best indicator for the presence of *P. aeruginosa* in grey water and for

its removal through the tested treatment technologies. Coliform bacteria are not, however, considered to adequately reflect the fate of non-bacterial pathogens, such as protozoa and viruses, in water (Leclerc *et al.*, 2001). Clostridia were not significantly correlated with *P. aeruginosa* removal in this study but have been proposed as a suitable indicator for the inactivation and removal of enteric viruses in drinking water treatment (Payment and Franco, 1993) and have been significantly correlated with the presence of *Giardia* cysts and *Cryptosporidium* oocysts in surface waters (Payment and Franco, 1993; Ferguson *et al.*, 1996; Brookes *et al.*, 2005). Clostridia removal by the constructed wetlands was low, compared to *E. coli* and Enterococci (Figures 1 and 2), suggesting a lower potential for removal of protozoan and viral pathogens than bacterial pathogens by these treatment technologies. Clostridia were effectively removed by the MBR and pathogenic protozoa would not be expected to pass through the membrane due to their size. Viruses, however, will pass through standard 0.4 µm pore size membranes (Madaeni, 1999), and would be pathogens of concern in membrane-treated grey water effluents.

The level of grey water treatment and/or disinfection used in practice should be determined by risk assessment of the potential for pathogen transmission from grey water reuse applications. The different risks associated with urban water reuse applications are reflected in many standards/guidelines, with an increasing likelihood of public exposure typically attracting more stringent criteria. Disinfection of all treated effluents would be required to ensure compliance with the most stringent microbiological standards for reuse. The presence of pathogenic protozoa and/or viruses in treated grey water effluent destined for reuse will impact the health risks associated with reuse and affect both the type and degree of disinfection required to ensure pathogen-free water for reuse. The quality of the treated effluents will impact on their subsequent disinfection and the potential for regrowth of bacteria following treatment. The superior removal of organics by the MBR and VFRB reduces the potential for regrowth and reduces the chemical disinfectant demand (Friedler *et al.*, 2006). The neutral pH values and low ammonia concentrations of the treated effluents would be suitable for disinfection by chlorine. Following chlorine disinfection, Friedler *et al.*

(2006) reported no regrowth of heterotrophic or faecal coliform bacteria in MBR treated grey water effluent.

3.4.5. Energy consumption

The energy consumption of pilot scale systems is not wholly representative of the energy required for full scale systems. The treatment technologies tested all used at least one electrical device for their operation. The calculated energy consumption of the VFRB was the lowest, at 0.4 $kW.h.m^{-3}$, while energy consumption of the other treatment systems was up to an order of magnitude greater. The VFRB required the lowest energy as it was fed intermittently, with the pump operational for less than one hour per day.

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CHAPTER 4:
CHLORINE DISINFECTION OF GREY WATER FOR REUSE: EFFECT OF ORGANICS AND PARTICLES

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4. CHLORINE DISINFECTION OF GREY WATER FOR REUSE: EFFECT OF ORGANICS AND PARTICLES

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ABSTRACT

Adequate disinfection of grey water prior to reuse is important to prevent the potential transmission of disease-causing microorganisms. Chlorine is a widely utilised disinfectant and as such is a leading contender for disinfection of grey water intended for reuse. This study examined the impact of organics and particles on chlorine disinfection of grey water, measured by total coliform inactivation. The efficacy of disinfection was most closely linked with particle size. Larger particles shielded total coliforms from inactivation and disinfection efficacy decreased with increasing particle size. Blending to extract particle-associated coliforms (PACs) following chlorine disinfection revealed that up to 91% of total coliforms in chlorinated grey water were particle associated. The organic concentration of grey water affected chlorine demand but did not influence the disinfection resistance of total coliforms when a free chlorine residual was maintained. Implications for urban water reuse are discussed and it is recommended that grey water treatment systems target suspended solids removal to ensure removal of PACs prior to disinfection.

4.1. INTRODUCTION

Grey water is defined as all flows exiting an urban building, excluding toilet water. Grey water can be considered a suitable candidate for reuse because it is consistently produced and is available onsite for reuse. Applications for the reuse of grey water include toilet flushing and garden irrigation. Grey water can be further classified as low load and high load in terms of organic strength. Low load grey water excludes the more polluted kitchen and laundry wastewater (Friedler, 2004). Treatment of grey water can

range from simple coarse filtration (March *et al.*, 2004) to advanced biological treatment (Nolde, 2005). Previous studies have suggested that biological processes should be prefered due to the high levels of organics in the water (Nolde, 1999; Jefferson *et al.*, 2004). Biological grey water treatment technology options for grey water reuse include: membrane bioreactor (Jefferson *et al.*, 2000), rotating biological contactor (Nolde, 1999; Friedler *et al.*, 2005) or constructed wetland (Dallas and Ho, 2005). All these biological systems have been capable of meeting a 10 $mg.L^{-1}$ BOD standard. The major differences between each technology has been the level of suspended solids and microorganism removal. In comparison, direct physical processes are common at very small scale and have been shown to remove solids but are less effective for organics removal (Jefferson *et al.*, 2004; Ramon *et al.*, 2004).

Suspended solids, or particles, in low load grey water originate from particulate matter shed from the human body, hygiene products, or sloughed biofilm from collection pipework, and are therefore expected to be predominantly organic in composition. The mechanisms by which microorganisms become associated with particles in grey water is not understood, however, microorganisms on the surface of the human body may be shed along with skin material during washing, forming a particle with associated microorganisms. The sloughing of biofilm, formed in grey water collection pipework, is another potential source of particle-associated microorganisms.

Indicator bacteria (coliforms, *Escherichia coli* and enterococci) are consistently detected in grey water (Ottoson and Stenström, 2003), demonstrating the potential for a range of enteric pathogenic bacteria (e.g. Salmonella, Campylobacter), protozoa (e.g. Cryptosporidium, Giardia) and viruses (e.g. rotavirus, norovirus) to persist in grey water. Indeed, prevous studies have isolated the opportunistic pathogens *Pseudomonas aeruginosa* and *Legionella pneumophilia* from grey water (Casanova *et al.*, 2001; Birks *et al.*, 2004). Microbiological standards or guidelines for urban water reuse vary worldwide. The California State Title 22 guidelines state that total coliforms should not exceed 2.2 $CFU.100mL^{-1}$ as an average over a seven-day period. The USEPA guidelines specify that faecal coliforms and viable pathogens be non-detectable in water intended for urban reuse. The presence of pathogens in grey water and their potential

transmission via reuse applications, as well as the existence of water quality standards, dictate that effective disinfection of grey water prior to reuse is essential. Coliform bacteria are useful as indicators, providing a measure of disinfection efficacy, however, their suitability as indicators for the disinfection of specific pathogens, particularly viruses and protozoa, is questionable. The importance of investigating the inactivation of coliform bacteria as a measure of disinfection success is stipulated by their inclusion in water reuse standards.

Chlorine is a widely utilised disinfectant, and as such, is a leading candidate for disinfection of grey water intended for reuse. The precise mechanism of microorganism inactivation by chlorine has not been fully elucidated. Studies have shown, however, that the bacterial cell membrane undergoes changes in permeability in the presence of chlorine and that the membrane is an important factor in determining bacterial resistance to chlorine disinfection (Venkobachar *et al.*, 1977; Virto *et al.*, 2005). Existing knowledge about the application of chlorine in associated fields has shown that suspended solids, or particles, and organics in wastewater are able to provide protection to microorganisms. Organic content in wastewater creates chlorine demand, reducing the availability of free chlorine for disinfection (LeChevallier *et al.*, 1981). March *et al.* (2004) reported a reduction in free residual chlorine in chlorinated grey water of 36 $mg.L^{-1}$ after 12 hours. It has been suggested that the presence of organics in water provide protection to bacteria through stabilisation of the cell membranes, restricting access of chlorine to key cellular components for inactivation (Virto *et al.*, 2005).

Microbial aggregates or microorganisms attached to or embedded in particles have been shown to demonstrate increased resistance to inactivation by chlorine compared to non-attached, free-swimming microorganisms (LeChevallier *et al.*, 1984; Dietrich *et al.*, 2003; Bohrerova and Linden, 2006). Dietrich *et al.* (2003) reported, however, that chlorine is capable of penetrating particles in wastewater by radial diffusion. They demonstrated that the degree to which chlorine penetrates particles in wastewater is influenced by a variable initial chlorine concentration ($mg.L^{-1}$) at a fixed dose ($mg.min.L^{-1}$). Chlorine penetration of particles up to 145 µm was reported. Greater chlorine penetration into wastewater particles was observed with increasing initial

chlorine concentration indicating that chlorine application could be tailored to penetrate particles of known size in order to achieve inactivation of particle-associated coliforms.

The aim of this study was to assess the impact of water quality, specifically organics and particles, on the chlorine disinfection of grey water. Knowledge of how these grey water characteristics limit disinfection informs selection of appropriate grey water treatment technologies to ensure adequate and cost-effective disinfection for reuse.

4.2. MATERIALS AND METHODS

4.2.1. Grey water characteristics

Grey water was collected from bathroom sinks, baths and showers of 18 specially plumbed student flats on Cranfield University campus. The grey water was collected by gravity in an underground sump and then pumped into a continuously circulating holding tank (1400L capacity). Grey water samples used in disinfection experiments were collected via a submersible pump (KP150, BSS) held in this tank. Samples were collected in sterile autoclaved polypropylene containers and either used immediately or stored at 5±1°C and analysed within 8 hours.

Biological and chemical oxygen demand (BOD, COD), total suspended solids (TSS) and total coliforms were measured routinely between November 2004 and July 2005. Particle size and chlorine demand measurements were carried out on random grab samples. BOD_5 was determined by the 5 day incubation method (APHA, 1998), total COD was analysed using the Merck spectroquant method, and total suspended solids were calculated according to standard methods (APHA, 1998). Measurement of particle size distributions was carried out using a Mastersizer 2000 (Malvern, UK). For each measurement of particle size distribution five repetitions were carried out using a measurement and background measurement time of 25s and a refractive index of 1.52. The manufacturer specifies a minimum and maximum detection limit of 0.2 µm and 2000 µm, respectively. The volume weighted mean D[4,3] was used to represent the

particle size distribution. D10, D50, and D90 values refer to the diameter below which lie a percentage of the total volume of all particles in the sample. Free chlorine demand was determined using the APHA Chlorine demand/requirement 2350B and DPD Ferrous Titrimetric 4500-Cl E methods (APHA, 1998), with a 30 minute contact time.

To assess the impact of organic content and particle size on grey water disinfection, grey water samples collected from the student flats were manipulated. The types of grey water used in this study are outlined in Table 4.1.

Table 4.1. *Description of grey water types used in the study.*

	Type of grey water	Description
A	Grey water	Unmodified grey water, collected via pump from holding tank.
B	Synthetic grey water	Shampoo, shower gel, and vegetable oil (20:20:1) in deionised water
C	Grey water + modified organics	Grey water with added synthetic grey water concentrate
D	Grey water + modified particles	Grey water settled into different particle size fractions.

4.2.2. Preparation of glassware and chlorine dosing solutions

All glassware was acid washed, rinsed three times in deionised water and dried before use. Chlorine dosing solutions were prepared in deionised water with sodium hypochlorite on the day of use. Chlorine dosing solutions were prepared at different concentrations to allow the addition of a consistent volume (<2% of total volume) to water samples to provide the desired initial chlorine concentration. The sodium hypochlorite was titred weekly according to APHA standard methods (APHA, 1998).

4.2.3. Chlorine inactivation experiments

All water samples and solutions were allowed to equilibrate to room temperature (18 ± 1°C) prior to their use in the chlorine inactivation experiments. Chlorine dosing solution

was added to a Pyrex bottle with PTFE-lined screw cap (Fisher Scientific) containing phosphate buffer at pH6.9±0.1 and the water sample. The bottle was filled to the top with the water sample, mixed, and kept in darkness at 18 ± 1°C. After 30 minutes, chlorinated samples were mixed and transferred to sterile bottles containing 1mL of sodium thiosulphate at a concentration sufficient to neutralise the initial chlorine added to the sample (Environment Agency, 2002). The neutralised samples were immediately stored at 4 ± 1°C for a maximum of 2 hours prior to total coliform enumeration. Total coliforms were enumerated on membrane lactose glucuronide agar (Oxoid) following standard membrane filtration methods (Environment Agency, 2002). Confirmation of presumptive total coliforms was performed according to standard methods (Environment Agency, 2002).

4.2.4. Particles and chlorine disinfection

Grey water fractions of varying particle size distributions (D) were created by collecting grey water (A) in a 10L settling container with rubber bungs at 2L intervals. The settling process created a gradient from the smallest particles at the top of the container to the larger particles at the bottom. Five separate 2L fractions were then extracted along the gradient providing grey water with a range of particle size distributions. Settling times from 10 minutes to 18 hours were used to achieve desired particle size distributions. Particle size, TSS and COD were measured for each fraction. Initial chlorine concentrations of 10, 20, 40 and 80 $mg.L^{-1}$ were applied to each fraction for a set contact time of 30 minutes. Free chlorine was neutralised using sodium thiosulphate and total coliforms were enumerated.

4.2.5. Particle-associated coliforms and chlorine contact time

500mL portions of a grey water (A) sample were subjected to a set 20 $mg.L^{-1}$ initial chlorine concentration over different contact time periods between 10 and 120 minutes. This ensured a free chlorine residual of more than 5 $mg.L^{-1}$ for the longest contact time of 120 minutes. Following chlorination the grey water was neutralised with sodium thiosulphate. 120mL was used for enumeration of total coliforms. The remainder was

added to a heat sterilised blender (KitchenAid Blender, model no. 5KSB52B) and blended at low speed (4000rpm) for 60 seconds. This has been shown to be an effective method for particle-associated coliform (PAC) extraction from wastewater particles with minimal impact on coliform viability, compared to other methods of physical or chemical extraction (Örmeci and Linden, 2005). Additional chemical extraction by EGTA or Camper's solution (Camper *et al.*, 1985) was found not to improve coliform recovery from grey water compared to blending alone and so was not used. The blender was rinsed five times with deionised water and sterilised by the addition of boiling water between each blended sample. A control of sterile deionised water was run with each batch of experiments to ensure the effectiveness of the sterilisation method. Total coliforms were enumerated following blending.

4.2.6. Organics and chlorine disinfection

Grey water was manipulated in terms of organic content by the addition of a synthetic grey water (B) concentrate to both real grey water (A) and settled grey water (D) to create grey water fractions of different organic concentration (C), as measured by Merck spectroquant TOC cell test (APHA, 1998). Particle size distribution, TSS and free chlorine demand (30 min contact time) of each fraction were measured. The initial chlorine concentration required to achieve a standard 1 $mg.L^{-1}$ free chlorine residual for each fraction was calculated from the chlorine demand data. Following a chlorine contact time of 30 minutes, total coliforms were enumerated from each fraction. The chlorine disinfection was performed in triplicate for each grey water fraction.

4.3. RESULTS AND DISCUSSION

4.3.1. Grey water characteristics

BOD and COD concentrations for the raw grey water (A) were 20 and 86 $mg.L^{-1}$, respectively (Table 4.2.). These are low when compared with those of fresh samples from separate grey water streams. Jefferson *et al.* (2004) reported 129 to 155 $mg.L^{-1}$

Table 4.2. *Grey water characteristics.*

	BOD ($mg.L^{-1}$)	**COD ($mg.L^{-1}$)**	**TOC ($mg.L^{-1}$)**	**TSS ($mg.L^{-1}$)**	**Total coliforms ($log_{10}CFU.100mL^{-1}$)**	**Particle size (Volume weighted mean, µm)**	**Particle size (D10, µm)**	**Particle size (D90, µm)**	**Free Cl demand ($mg.L^{-1}$)**
Mean	20	86	49	29	5.26	286	13	763	9.8
Standard deviation	6	23	13	34	0.80	142	8	304	0.5
Min.	8	33	30	4	4.00	104	7	312	8.9
Max.	34	138	65	164	7.06	505	30	1099	10.5
No. samples	74	47	5	77	36	7	7	7	8

Min.: Minimum; Max.: Maximum

BOD and 367 to 587 $mg.L^{-1}$ COD in fresh grey water samples from the three low load individual grey water streams: hand basin, shower, and bath water. The grey water was also of low strength when compared to published data for mixed low load grey water. Friedler *et al.* (2005) reported BOD, COD and TSS values of 59, 158 and 43 $mg.L^{-1}$, respectively, for grey water collected from hand basins, showers and baths. The numbers of total coliforms, 5.26 $\log_{10}CFU.100mL^{-1}$ were in the mid-range of low load grey waters according to literature data collated by Ottoson and Stenström (2003) who reported values ranging from 1.8 to 7.4 $\log_{10}CFU.100mL^{-1}$ total coliforms.

Particle size analysis revealed that the maximum particle size of the grey water was ≥2000 μm, the upper limit for detection by the apparatus. On average, particles larger than 13μm made up 90% of the total particle volume, and particles larger than 763μm constituted 10% of the total volume. The data is similar to that of Ramon *et al.* (2004) who studied low load grey water with a similar TSS content. They reported that particles larger than 5μm constituted 95% of the total particle volume but less than 1% of the total particle number. Mean values with standard deviation for pH, temperature, ammonia, and turbidity in the grey water (A) were pH7.3±0.2, 16.8±4.7°C, 1.6±1.7mg/L, and 19.6±16.1NTU, respectively.

The free chlorine demand exerted by the grey water was approximately 10 $mg.L^{-1}$ over a 30 minute contact period for the samples tested. Chlorine demand measurements revealed that the raw grey water (A) created 0.19 mg free Cl demand/mg TOC compared to the synthetic grey water (B), which created 0.03 mg free Cl demand/mg TOC (data not shown). This indicates that the human-derived organics and microorganisms in real grey water create the greatest proportion of free chlorine demand per mg TOC compared to the organics from hygiene products alone.

4.3.2. Chlorine inactivation of indicator bacteria in grey water

Inactivation of total coliforms in raw grey water (A) by chlorine, revealed a typical wastewater disinfection curve with a lag phase, linear inactivation, and a tailing phase (Figure 4.1.). The lag phase, up to 1 $mg.L^{-1}$, is due to the instant chlorine demand

created by oxidisable compounds, which render chlorine ineffective for disinfection (Metcalf and Eddy, 2003). In grey water, antioxidants from commercial hygiene products contribute to this chlorine demand by reducing hypochlorite to chloride ions (March *et al.*, 2005). The linear inactivation response curve between 1 and 5 $mg.L^{-1}$ can be attributed to the inactivation of free-swimming coliforms, and the tailing effect to the shielding of coliforms by particles. This tailing effect has been previously reported in both ultraviolet (UV) and chlorine disinfection systems (Emerick *et al.*, 1999; Dietrich *et al.*, 2003). The persistence of total coliforms at initial chlorine concentrations of up to 80 $mg.L^{-1}$ demonstrates extremely robust particle shielding of coliforms in grey water.

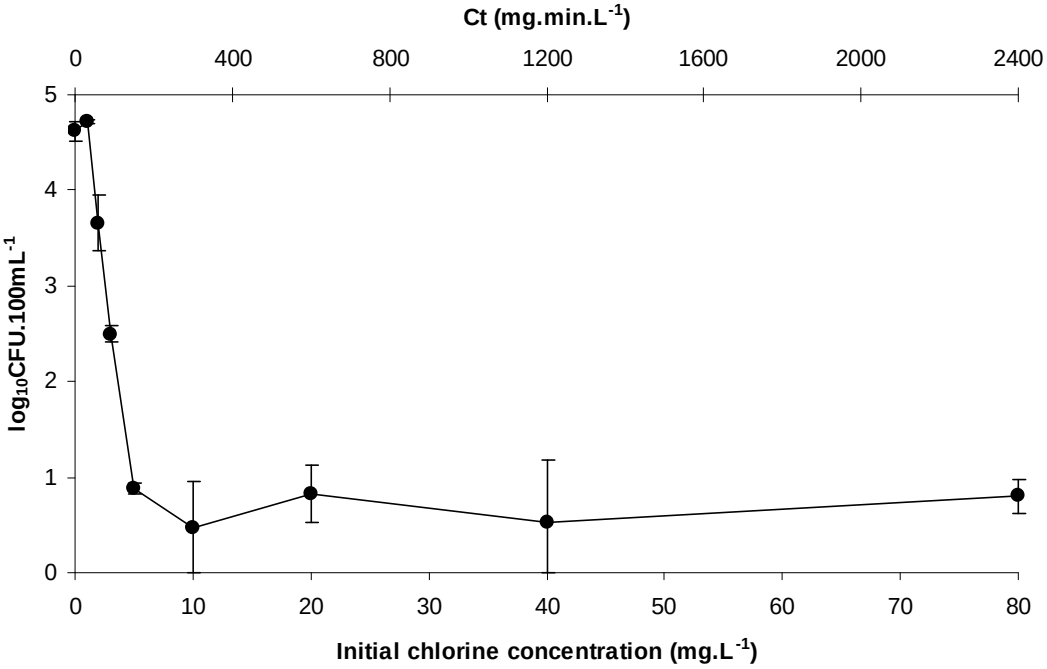

Figure 4.1. *Inactivation of total coliforms by chlorine (30 min contact time) in raw grey water (A). Data are mean values with standard deviation, n=3.*

4.3.3. Impact of particle size on chlorine disinfection

The mean particle sizes of the different grey water fractions represent the shift in particle size distribution between the fractions (Table 4.3.). The maximum particle size exceeded the detection limit of 2000 µm for a number of the grey water fractions. These very large particles did not constitute a large proportion of the total particles, however,

Table 4.3. *Particle size distribution details of grey water fractions.*

Volume weighted mean (D[4,3], µm)	D10 (µm)	D50 (µm)	D90 (µm)	Max. size (µm)
34	6	18	64	724
38	6	19	74	724
67	8	42	165	479
81	8	42	178	≥2000
159	15	98	396	1096
177	15	130	414	955
182	15	103	458	1660
201	16	116	491	≥2000
230	18	175	536	1096
288	20	190	693	≥2000
405	32	323	897	≥2000
415	38	343	882	≥2000
448	42	371	961	≥2000
512	55	431	1076	≥2000

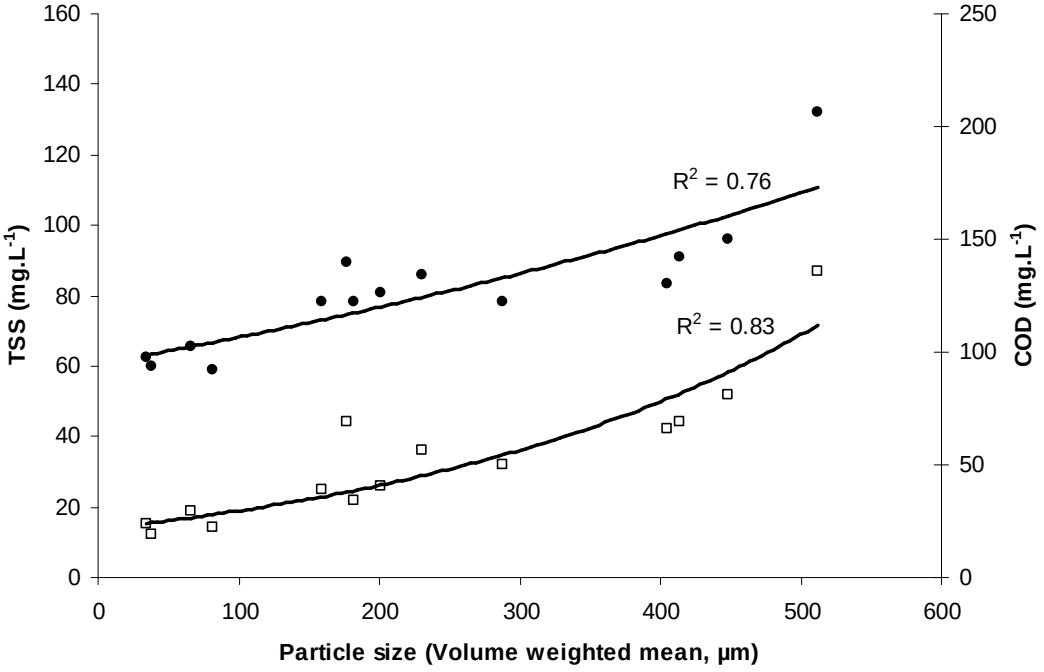

Figure 4.2. *Total suspended solids (TSS, □) and chemical oxygen demand (COD, ●) for particle size fractions represented by the volume weighted mean.*

because in the fraction with the highest mean particle size, the D90 value demonstrates that only 10% of the total volume of particles were larger than 1076 µm in size. Analysis of the grey water in relation to particle size revealed that as the mean particle size increased from 34 to 512 µm the COD increased from 92 to 206 $mg.L^{-1}$ and the TSS from 12 to 87 $mg.L^{-1}$ (Figure 4.2.). TSS was most closely correlated with mean particle size, with an R^2 value of 0.83 compared to 0.76 for COD.

Total coliform survival in chlorinated grey water was greater with increasing particle size. Following chlorine disinfection at an initial concentration of 20 $mg.L^{-1}$, for example, total coliform survival rose from -4.5 to -3.1 with an increase in mean particle size from 34 to 512 µm, (Figure 4.3.). A similar trend of greater coliform survival with increased particle size was observed at all initial chlorine concentrations. The applied initial chlorine concentration also impacted coliform survival. Greater initial chlorine concentrations reduced coliform survival at all particle size distributions. Total coliform survival decreased from -2.9 to -3.8 with initial chlorine concentrations from 10 to 80 $mg.L^{-1}$ at the highest mean particle size of 512 µm, for example. Complete coliform inactivation was achieved at mean particle sizes below 177 µm and 230 µm with 40 and 80 $mg.L^{-1}$ initial chlorine, respectively. Coliform survival was observed at the lowest mean particle size of 34 µm with 10 and 20 $mg.L^{-1}$ initial chlorine.

The trendlines in Figure 4.3. converge towards the higher mean particle sizes indicating that the effect of increased initial chlorine concentration on coliform inactivation was limited at the larger particle size fractions. Total coliform survival decreased from -2.9 to -3.8, a difference of 0.9, with increasing initial chlorine concentration at the mean particle size of 512 µm. This compares to a decrease from -3.6 to -5.5, a difference of 1.9, at the lower mean particle size of 230 µm. Particle penetration and inactivation of coliforms clearly increased with increasing initial chlorine concentration, however, the larger particles in grey water were able to provide protection to coliforms even at high initial chlorine concentrations of 80 $mg.L^{-1}$.

Longer chlorine contact times resulted in less total coliform survival in grey water. To illustrate, with the addition of 20 $mg.L^{-1}$ initial chlorine, total coliform counts reduced

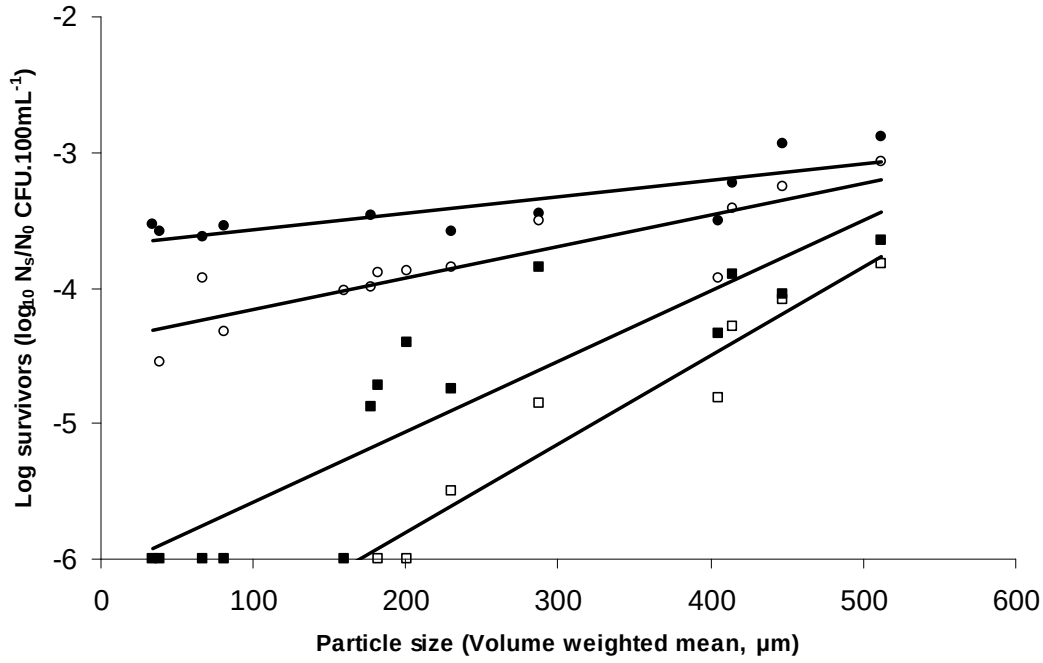

Figure 4.3. *Proportion of surviving total coliforms against particle size in manipulated grey water, following disinfection at initial chlorine concentrations of 10 (●), 20 (○), 40 (■), and 80 (□) mg.L^{-1}. Cases of zero coliform survival have a value of -6.*

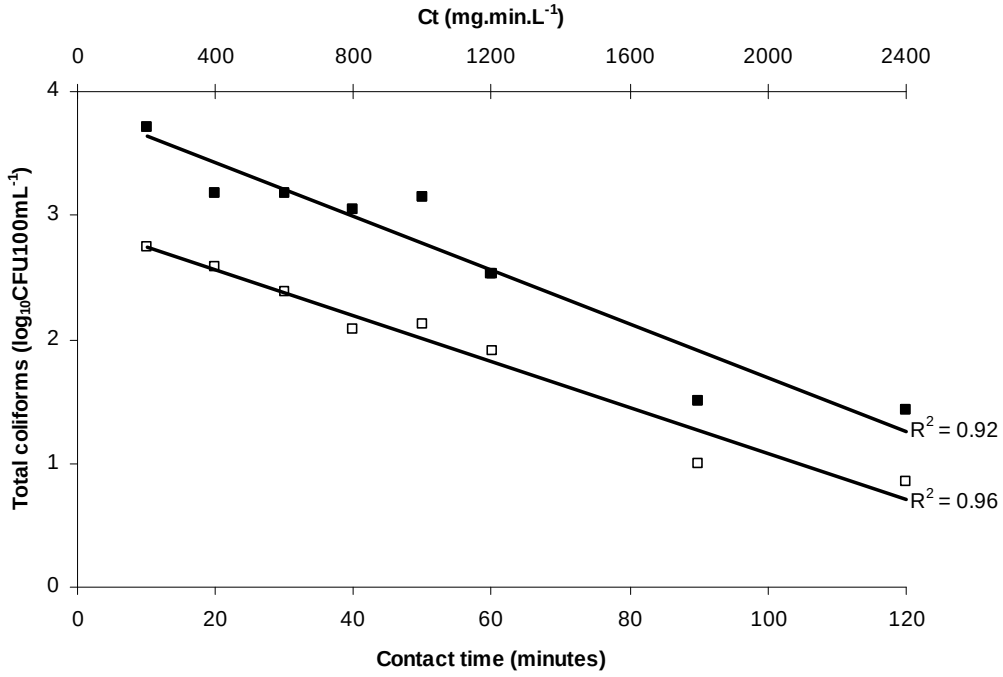

Figure 4.4. *Total coliform concentration in grey water against chlorine contact time. Initial chlorine concentration was 20 mg.L^{-1}, free chlorine was measured as 4 mg.L^{-1} after 120 min contact time. Data shows post-chlorination blended (■) and non-blended (□) grey water samples.*

from 2.74 to 0.85 $\log_{10}$CFU.100mL^{-1} with an increase in contact time from 10 to 120 minutes (Figure 4.4.). Higher total coliform counts were observed for the blended samples, which included extracted PACs, across the range of chlorine contact times. Blending of unchlorinated grey water revealed that 36% of the total coliforms were particle-associated and were not enumerated without extraction by blending. At the shortest chlorine contact time of 10 minutes, blending increased measured total coliforms by nine times, with PACs now making up 91% of the total coliform count. By the longest contact time, blending resulted in an increase in total coliforms of just four times, demonstrating that increasing chlorine contact time resulted in greater particle penetration by chlorine and subsequently, greater inactivation of PACs. PACs were still present at the highest contact time of 120 minutes, however, at which time complete penetration of grey water particles by chlorine was not achieved.

The resistance of particle-associated bacteria to chlorine disinfection, as shown in the current study, has been reported in a variety of circumstances in the literature. For instance, LeChevallier *et al.* (1981) showed that particles afforded protection to bacteria from inactivation in chlorinated surface water. They reported that blending to shear particles increased standard plate count bacteria by up to five times. Berman *et al.* (1988) demonstrated the shielding of coliform bacteria from chlorine disinfection in primary sewage effluent by particles greater than 7 µm, while Bohrerova and Linden (2006) reported increased resistance to chlorine disinfection of Mycobacterium aggregates above 41 µm, spiked into secondary effluent.

Particle shielding of bacteria has also been demonstrated in UV disinfection systems where coliform bacteria associated with particles larger than 20 µm in wastewater have been reported to show resistance to UV disinfection (Madge and Jensen, 2006). Particles below 10 µm are not considered to provide shielding to coliform bacteria (Emerick *et al.*, 1999). In the case of viruses, however, which are several orders of magnitude smaller than bacteria, certain types of particles of less than 2 µm are considered capable of shielding from UV light (Templeton *et al.*, 2005).

Dietrich *et al.* (2003) demonstrated, using wastewater, that the minimum particle size capable of shielding associated coliforms from chlorine increased with increasing initial chlorine concentration, as a result of increased particle penetration at higher initial chlorine concentrations. These experiments demonstrate that the particle size at which PACs in grey water are shielded from chlorine is variant with the initial chlorine concentration. Greater initial chlorine concentrations increased the particle size required for coliform shielding to take place. The lowest mean particle sizes with associated coliforms in grey water were 34, 177, and 230 µm with initial chlorine concentrations of 20, 40, and 80 $mg.L^{-1}$, respectively.

4.3.4. Impact of organics on chlorine disinfection

In order to examine the impact of organics on chlorine disinfection of grey water it was necessary to vary the initial chlorine concentration to provide a standard free chlorine concentration of 1 $mg.L^{-1}$. This meant that the chlorine demand created by the organics was eliminated as a factor in the experiment, thus allowing sole focus on whether the organics provide an actual protective effect to the microorganisms in grey water, rather than simply a chlorine demand effect.

Total coliform survival did not increase with increasing TOC concentration for the grey water (A) or settled grey water (D) (Figure 4.5.). Indeed, coliform kill appears to increase with increasing TOC. Total coliform survival was 2.28 $log_{10}CFU.100mL^{-1}$ at a TOC of 65 $mg.L^{-1}$ for the unmodified grey water, compared to 1.86 $log_{10}CFU.100mL^{-1}$ at a TOC of 153 $mg.L^{-1}$. However, the standard error present in these data make it an unreliable trend. This trend may be due to the higher initial chlorine concentration required to meet the increasing chlorine demand with increasing TOC. If the free chlorine demand of the TOC was not instantaneous, a greater initial free chlorine concentration may have resulted in increased coliform inactivation.

Organics in water have been shown to affect the efficacy of chlorine disinfection; however, this is commonly attributed to an increase in chlorine demand and resultant reduction in free chlorine available for disinfection (LeChevallier *et al.*, 1981). Virto *et*

al. (2005) investigated the effect of an organic source, trypticase soya broth (TSB), on bacteria undergoing chlorine disinfection. They demonstrated a protective effect of organics that was not simply due to an increased chlorine demand because higher free chlorine residual was present than was required for inactivation of the bacteria in deionised water. They suggested that organics in water increase the resistance of bacteria to chlorine by stabilizing the bacterial membranes and restricting chlorine access to cellular components to cause inactivation. The grey water used in this study contained significant levels of organics (Table 4.2.) and therefore any increased resistance due to the stabilizing effect of organics would already exist. The data here shows that this protective effect is not enhanced with the presence of additional organics in grey water.

Table 4.4. *Measured water quality characteristics of unmodified grey water (A) and settled grey water (D) without addition of synthetic grey water concentrate. Data are mean values, n = 2.*

	COD ($mg.L^{-1}$)	**TOC ($mg.L^{-1}$)**	**TSS ($mg.L^{-1}$)**	**Mean particle size (volume weighted, µm)**
Grey water	98	65	27	149
Settled grey water	34	24	24	30

Greater coliform survival, of almost 2 log units, was observed for the unmodified grey water than the settled grey water, irrespective of TOC concentration (Figure 4.5.). The additional TOC had no impact on coliform resistance to chlorine disinfection. Particle size was the clear differentiator between the two samples, which had similar TSS values (Table 4.4.). The unmodified grey water had a d0.9 particle size of 412 µm compared to 65 µm for the settled grey water. This suggests that high levels of organics in grey water play no role in protecting coliforms from chlorine disinfection and further points to particle size as the key vehicle for coliform resistance to chlorine disinfection in grey water.

4.4. IMPLICATIONS FOR URBAN REUSE

The results presented in this paper have demonstrated two key points with respect to grey water treatment for reuse: (1) the organic concentration affects disinfectant demand but does not influence microbial resistance to inactivation, and (2) the effectiveness of chlorine for disinfection is most closely linked to the particle size of the material in grey water.

The grey water collected in this real system contained particles of 2000 µm diameter or greater. These particles were likely formed by microbial aggregation, flocculation, and biofilm formation in the collection pipes and storage container. Importantly, these particles contained embedded and attached bacteria. Data from this study showed that 36% of coliform bacteria in the grey water were particle associated. The proportion of PACs rose to as much as 91% following addition of chlorine demonstrating that chlorine was effective for the inactivation of free, non-attached coliforms in grey water but had limited efficacy for the inactivation of PACs in grey water. Coliforms associated with the larger particles in grey water were most resistant to inactivation, preventing complete disinfection of coliform bacteria. As a result, direct chlorine disinfection of the grey water used in this study did not meet stringent international microbiological standards for urban water reuse. The California State Title 22 requirement of 2.2 total coliforms per 100mL for unrestricted urban reuse was unattainable at initial chlorine concentrations of up to 80 $mg.L^{-1}$. Initial chlorine concentration and contact time can be increased to improve particle penetration and inactivation of PACs in grey water, however, high chlorine concentrations and long contact times may not be practical options for grey water reuse.

The existence of PACs has important ramifications for disinfected effluents intended for reuse. Coliform enumeration by standard techniques such as membrane filtration and MPN method will record one particle, containing any number of PACs, as one coliform, or colony forming unit. Consequently, a disinfected grey water, meeting the appropriate standards, may be deemed fit for reuse despite harbouring up to nine times the measured number of total coliforms, as PACs. The extent to which specific pathogens become

particle associated in grey water is not known, although it can be assumed that pathogens will become particle associated in the same manner as coliforms. Pathogenic particle-associated microorganisms in grey water and the potential for regrowth may present a health risk upon reuse of chlorine-disinfected grey water (Dixon *et al.*, 1999).

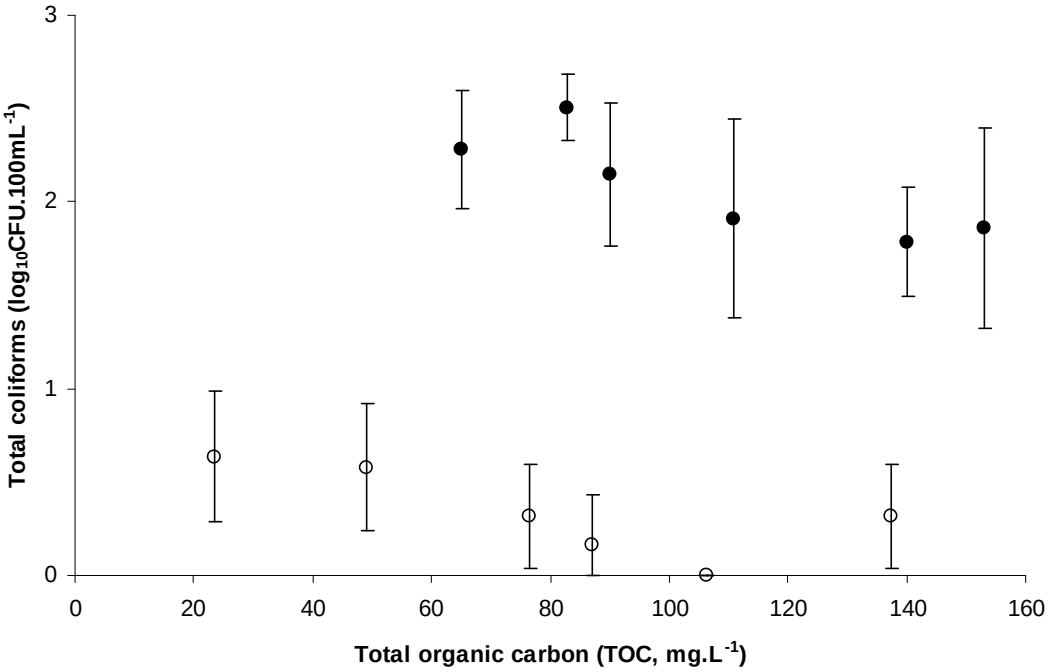

Figure 4.5. *Total coliforms against total organic carbon (TOC) in grey water (●) and settled grey water (○) supplemented with synthetic grey water, following disinfection at a free chlorine concentration of 1 mg.L^{-1}. Total coliform concentration in unmodified and settled grey water samples prior to disinfection was 5.4-5.7 and 4.0-4.4 log_{10}CFU.100mL^{-1}, respectively. Data are mean values with standard deviation, n=3.*

For this reason, removal of suspended solids, and therefore the larger particles from grey water, is a key requirement to achieve subsequent effective disinfection. March *et al.* (2005) reported a hotel in Mallorca utilising grey water to flush hotel toilets with a simple treatment stage involving coarse filtration (300 µm pore size) followed by the addition of chlorine at an initial concentration of 75 mg.L^{-1}. Samples tested for total coliforms were reported negative, provided a free chlorine residual was maintained. By limiting the maximum particle size to 300 µm and providing a high chlorine concentration to encourage particle penetration by chlorine and inactivation of PACs, complete disinfection of total coliforms in grey water was achieved, which is in

agreement with the data presented here. The drawback to this method of grey water treatment and reuse is that high levels of organics remain in the grey water. Grey water contains organic material contributed by personal hygiene products such as soaps or shampoos as well as human-derived organics, including skin and excreta (Ottoson and Stenström, 2003). This study indicates that organics in grey water do not limit disinfection provided the initial chlorine concentration is adjusted to meet any additional chlorine demand and maintain a free chlorine residual. However, organics increase the required initial chlorine concentration and cause chlorine decay resulting in a limited residence time post-chlorination before odour and regrowth become a problem (March *et al.*, 2005).

Simple coarse filtration treatment systems will remove the largest particles in grey water, improving the efficacy of chlorine disinfection. Particles below the filter pore size and the majority of the organics will remain, however, requiring high initial chlorine concentration and contact time to ensure penetration of remaining particles, to meet the chlorine demand presented by the organics, and also to account for chlorine decay over time. Finer physical filtration of grey water, such as by ultrafiltration, can further reduce particle size in the effluent so as to be insignificant in terms of particle shielding from disinfection. Fine filtration can act as a disinfection step in itself but a disinfectant residual is still regarded as important to prevent microbial regrowth in the effluent. Dissolved organics can remain in the effluent following this treatment method (Ramon *et al.*, 2004), providing chlorine demand and nutrients for bacterial regrowth. Biological treatment systems, such as constructed wetlands, biological filters, rotating biological contactors can potentially provide excellent organics removal performance, with effluent BOD values below 5 $mg.L^{-1}$ (Nolde, 1999; Dallas and Ho, 2005; Friedler *et al.*, 2005). Such treated grey water effluent would require substantially lower initial chlorine concentration to maintain a residual. The potential for particulate matter, containing particle-associated microorganisms, to exit these treatment systems would be of concern, however. Should such particles exist in the effluent, there is the risk that the lower initial chlorine concentrations used to maintain the required residual may be insufficient to ensure penetration of these particles by chlorine. Membrane bioreactors, combining biological treatment with physical filtration, combine the excellent organics

removal of biological processes with the particulate removal and disinfection capability of physical filtration systems for grey water treatment (Jefferson *et al.*, 2000). A low chlorine concentration is thus sufficient to maintain a residual following treatment.

4.5. CONCLUSIONS

The chlorine disinfection of grey water in this study was affected by the presence of organics and particles. Organic concentration in grey water did not effect resistance of coliform bacteria to chlorine disinfection at a fixed free chlorine residual. Particles in grey water harboured particle-associated coliforms, which were highly resistant to chlorine disinfection, preventing adequate disinfection of grey water for reuse. For effective disinfection, grey water treatment facilities should target suspended solids removal to remove PACs prior to disinfection. Additional removal of organics is also beneficial, creating a low, stable chorine demand, and reducing the potential for microbial regrowth in the distribution system and, in the case of toilet flushing applications, the toilet cistern.

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CHAPTER 5:
ULTRAVIOLET (UV) DISINFECTION OF GREY WATER: PARTICLE SIZE EFFECTS

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5. ULTRAVIOLET (UV) DISINFECTION OF GREY WATER: PARTICLE SIZE EFFECTS

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ABSTRACT

The impact of water quality on the ultraviolet (UV) disinfection of grey water was investigated with reference to urban water reuse. Direct UV disinfection of grey water did not meet the stringent California State Title 22 criteria for unrestricted urban water reuse due to the presence of particulate material ranging from <1 to ≥2000 µm in size. Grey water was manipulated to produce fractions of varying particle size distributions and blending was employed post-disinfection to extract particle-associated coliforms (PACs). The efficacy of UV disinfection was found to be linked to the particle size of the grey water fractions. The larger particle size fractions with a mean particle size of 262 µm and above were observed to shield more coliforms from UV light than smaller particles with a mean particle size below 119 µm. Up to 70% of total coliforms in the larger particle size fractions were particle associated following a UV dose (fluence) of 260 $mJ.cm^{-2}$ and would remain undetected by standard coliform enumeration techniques. The organic concentration of grey water affected UV transmittance but did not influence microbial resistance to inactivation by UV light when a constant UV dose was applied. Implications for urban water reuse are discussed and recommendations made for grey water treatment to ensure removal of particle-associated indicator bacteria and pathogens prior to UV disinfection.

5.1. INTRODUCTION

Urban wastewater reuse is recognised as an important facet in addressing regional water supply shortages and in maintaining sustainable urban environments. Local, decentralised water reuse schemes involving individual and clusters of homes,

institutions, and commercial premises', are an attractive option as they minimise the need for additional distribution infrastructure (Anderson, 1996; Tchobanoglous and Angelakis, 1996). Grey water is a suitable candidate for decentralised water reuse schemes because it is consistently produced onsite. Grey water can be defined as all in-building wastewater flows with the exception of toilet wastewater. The separation of grey water from toilet wastewater is advantageous as it reduces the pollutant load, in terms of organics, pathogens, and toilet paper waste. Grey water can be further separated into 'light' or 'low load' grey water, which includes only bathroom grey water and excludes the more polluted kitchen and laundry grey water (Surendran and Wheatley, 1998; Friedler, 2004). The selection of grey water sources is typically determined by the water volume requirements of the reuse applications. Principal urban water reuse applications include toilet flushing and landscape irrigation.

Coliform bacteria and faecal indicator bacteria are commonly reported in grey water. Total coliform concentrations from 3.8 to 6.8 $\log_{10}.100mL^{-1}$ have been reported in bathroom grey water sources (Surendran and Wheatley, 1998; Jefferson *et al.*, 2005) while concentrations of up to 8.1 $\log_{10}.100mL^{-1}$ have been reported in grey water inclusive of kitchen and laundry wastewater (Casanova *et al.*, 2001; Ottoson and Stenström, 2003). The presence of faecal indicator bacteria *Escherichia coli* and Enterococci in grey water (Ottoson and Stenström, 2003) indicates the potential presence of faecally-transmitted pathogens in grey water. Thus, the potential for pathogen transmission from reuse applications means that adequate disinfection of grey water is critical. Such a requirement is reflected in water quality standards for urban reuse, although the specific consent levels vary worldwide. For instance, the California State Title 22 criteria of 2.2 total coliforms per 100mL over a 7-day median (USEPA, 2004) are are some of the most stringent microbiological standards for unrestricted urban reuse, which includes use for toilet flushing and irrigation of public areas such as parks and residential lawns. Less restrictive criteria for urban reuse apply elsewhere, for example, Costa Rican guidelines stipulate less than 100 faecal coliforms per 100mL (Dallas *et al.*, 2004), while guidelines in Germany are based on EU standards for recreational waters of less than 10000 total coliforms per 100mL (Nolde, 1999).

Ultraviolet (UV) light is perceived as an 'environmentally friendly' disinfection technology, which avoids the use of chemicals and is in keeping with the sustainable objectives of urban water reuse. However, particulate matter and organics in water can limit UV disinfection by attenuating UV light; reducing UV transmittance and the dose received by the targeted microorganisms. Particles can also 'shield' embedded microorganisms from UV light ensuring their persistence post-disinfection. The negative impact of particles on the efficacy of UV disinfection is well documented in wastewater (Qualls *et al.*, 1983; Parker and Darby, 1995; Madge and Jensen, 2006) and drinking water (Christensen and Linden, 2003; Liu *et al.*, 2007; Templeton *et al.*, 2007). The size at which particles offer protection from UV light varies with microorganism type. For example, the critical size at which particles are capable of shielding bacteria from UV disinfection has been reported as 10 µm (Qualls *et al.*, 1985; Emerick *et al.*, 1999), while particle shielding of viruses, which are orders of magnitude smaller than bacteria, can occur with particles of 2 µm or lower (Templeton *et al.*, 2005). The larger size of protozoan cysts/oocysts, compared to bacteria, are thought to require larger particle sizes of >25 µm for shielding from UV light to occur (Amoah *et al.*, 2005). Whilst the efficacy of UV disinfection has been shown to decrease with increasing particle size (Madge and Jensen, 2006), the impact of particle size on the quantity of microorganisms shielded from UV light is not well known. Grey water has been shown to contain very large particles of up to 2000µm (Chapter 4), potentially facilitating the shielding of significant numbers of microorganisms from disinfection by UV light. The number of microorganisms embedded within particles and shielded from UV light is important because it can lead to underestimation of actual counts by common enumeration techniques (Parker and Darby, 1995; Emerick *et al.*, 1999). Chemical and/or physical extraction methods can allow quantification of particle-associated microorganisms (Örmeci and Linden, 2005) which may be shielded from UV light.

This paper investigates the extent of particle-association of coliform bacteria in grey water and the relationship between particle size and the resistance of coliforms to UV disinfection. The impact of grey water treatment on UV disinfection efficacy is discussed and recommendations are made for the appropriate treatment of grey water prior to UV disinfection and reuse.

5.2. MATERIALS AND METHODS

5.2.1. Water sample collection

Grey water was collected from bathroom sinks, baths and showers of 18 specially plumbed student flats into a continuously circulating holding tank (1400L capacity). Grey water samples used in disinfection experiments were collected via a submersible pump (KP150, BSS) held in this tank. A horizontal-flow reed bed (HFRB), treated grey water fed at a constant flow rate of 480L.d^{-1}. The HFRB had a 6m^2 surface area and 0.7m depth, utilised a sand/soil/compost mixed media, and was planted with *Phragmites australis*. The hydraulic retention time was determined by lithium tracer study as 2.1d. Treated wastewater effluent was taken from Cranfield sewage works after the following treatment train: maceration, sedimentation, trickling filtration, tertiary nitrifying filtration, and clarification. Samples were collected in sterile autoclaved polypropylene containers and either used immediately or stored at 5±1°C and used within two hours.

5.2.2. Water quality analysis

Biological and chemical oxygen demand (BOD, COD), total suspended solids (TSS), turbidity were calculated according to standard methods (APHA, 1998). Measurement of particle size distributions was carried out using a Mastersizer 2000 (Malvern, UK). Five repetitions were carried out using a measurement and background measurement time of 25s and a refractive index of 1.52. The manufacturer specifies a minimum and maximum detection limit of 0.2 µm and 2000 µm, respectively. The volume weighted mean D[4,3] was used to represent the particle size distribution. D10, D50, and D90 values refer to the diameter below which lie a percentage of the total volume of all particles in the sample. Fractal dimensions were calculated from the raw output data of the Mastersizer, which was converted to provide the angle of each detector and the intensity of light at each detector by using a spreadsheet provided by Malvern Instruments (Malvern, UK).

Total coliforms and *Escherichia coli* were enumerated by most probable number (MPN) method using IDEXX Colisure and Quanti-Tray (Maine, USA) according to standard methods (APHA, 1998) and Enterococci were enumerated by standard membrane filtration technique on Slanetz and Bartley Agar. To include data with a value of less than one, microbial data is reported as $\log_{10}(y+1).100mL^{-1}$, with standard deviation.

5.2.3. UV inactivation experiments

UV light inactivation experiments and collimated beam operation were carried out as described by Bolton and Linden (2003). Water samples were allowed to equilibrate to room temperature (18 ± 1°C) prior to their use in the inactivation experiments. 300mL aliquots were added to a 20cm diameter petri dish, giving a 1cm sample depth, and were magnetically stirred by a sterile 2cm stirrer bar at 100rpm for 10 seconds prior to, and during, UV light exposure. Samples were exposed to 254nm UV-C irradiation from a collimated beam device (Wedeco AG, Germany) consisting of four low pressure UV lamps and a pneumatically controlled shutter. The applied UV fluence rate was determined by uridine actinometry in deionised water (DVGW, 1997) as $2.08mW.cm^{-2}$ and the UV dose (or fluence) was controlled by exposure time. When equivalent UV doses were required for different waters, exposure time was adjusted to correct for the UV transmittance and depth of the sample. UV_{254} absorbance of samples was measured through a 1cm quartz cell in a UV-Vis spectrophotometer. The spectrophotometer was not equipped with an integrating sphere. This could have resulted in an overestimation of UV absorbance, as light reflected off particles and not passing directly through the sample impacts the absorbance measurement (Christensen and Linden, 2003). The overestimation is expected to be between 25 and 46% for the grey water and treated wastewater effluent samples, based on data reported by Sheible *et al.* (1986) for wastewater effluents. Following UV light exposure, samples were transferred to sterile containers and used immediately or stored at 5 ± 1°C for a maximum of 2 hours prior to total coliform enumeration.

5.2.4. Extraction of particle-associated coliforms

A blending method was used to extract and disperse coliform bacteria associated with particles. Blending was carried out in a heat-sterilised blender (KitchenAid Blender, model no. 5KSB52B) and blended at low speed (4000rpm) for 60 seconds. The blender was rinsed five times with deionised water and heat-sterilised with boiling water between samples. A control sample of sterile deionised water was included in each set of tests to confirm that cross-contamination did not occur between blended samples. Low speed blending has been shown to be an effective method for particle-associated coliform (PAC) extraction from wastewater particles with minimal impact on coliform viability, compared to other methods of physical or chemical extraction (Örmeci and Linden, 2005). Additional chemical extraction by EGTA or Camper's solution (Camper *et al.*, 1985) was found not to improve coliform recovery compared to blending alone and so was not used. Subtraction of the non-blended coliform count from the blended coliform count gave the number of PACs and allowed calculation of the percentage of PACs as part of the total coliform population.

5.2.5. Particles and UV disinfection

Grey water fractions of varying particle size distributions were created by a settling process. Settling times from 10 to 60 minutes were used to achieve desired particle size distributions. Water quality measurements were taken for each fraction. A UV dose of 260 $mJ.cm^{-2}$ was applied to each fraction, selected from the UV dose-response data for grey water to ensure inactivation of dispersed coliforms and the survival of only those coliforms embedded within particles. Total coliforms were enumerated with and without extraction of PACs by blending.

5.2.6. Organics and UV disinfection

Grey water was manipulated in terms of organic content by the addition of a synthetic grey water concentrate, consisting of shampoo, shower gel, and vegetable oil (20:20:1) in deionised water, to create fractions of different organic concentration, as measured by

Merck spectroquant TOC cell test. Water quality measurements were taken for each fraction. Following a UV dose of 13 $mJ.cm^{-2}$, total coliforms were enumerated from each fraction, with and without blending to extract PACs.

5.3. RESULTS AND DISCUSSION

5.3.1. UV inactivation curves

The inactivation of indicator bacteria in grey water by UV light was great at low UV doses. At a UV dose of 5.8 $mJ.cm^{-2}$, for example, log_{10} inactivations were 3.2, 2.4, and 3.0 for total coliforms, *E. coli*, and Enterococci, respectively (Figure 5.1.). The application of higher UV doses to the grey water gave minimal additional inactivation of indicator bacteria. To illustrate, additional log_{10} inactivations of total coliforms, *E. coli*, and Enterococci, between the UV doses of 5.8 and 69 $mJ.cm^{-2}$, were just 0.9, <0.1, and 0.4, respectively. This tailing effect for the inactivation of indicator bacteria in grey water has previously been reported for the UV disinfection of waters containing particulate matter (Emerick *et al.* 1999; Loge *et al.*, 1999; Fenner and Komvuschara, 2005). Emerick *et al.* (1999) showed that the majority of coliform bacteria were inactivated at a UV dose below 40 $mJ.cm^{-2}$ in biologically treated wastewater effluents exposed to UV doses ranging from 5 to 400 $mJ.cm^{-2}$ and they defined the residual coliform population as those surviving a UV dose above 100 $mJ.cm^{-2}$. The initial linear inactivation response curves can be attributed to the inactivation of dispersed, non-attached bacteria, and the tailing phase to the shielding of those bacteria attached to, or embedded within, particulate matter in the grey water. A similar tailing effect has been reported for total coliforms in grey water (thesis chapter 4), following chlorine disinfection. Total coliforms persisted in the grey water following initial chlorine Ct doses of up to 2400 $mg.min.L^{-1}$. Following a UV dose of 277 $mJ.cm^{-2}$, total coliforms were enumerated at a mean concentration of 1.0±0.2 $log_{10}MPN.100mL^{-1}$ in the grey water, whereas *E. coli* and Enterococci were not detected. Total coliform survival continued a gradual decline with increasing UV dose in the tailing region of the inactivation curve, indicating some level of particle penetration by UV light.

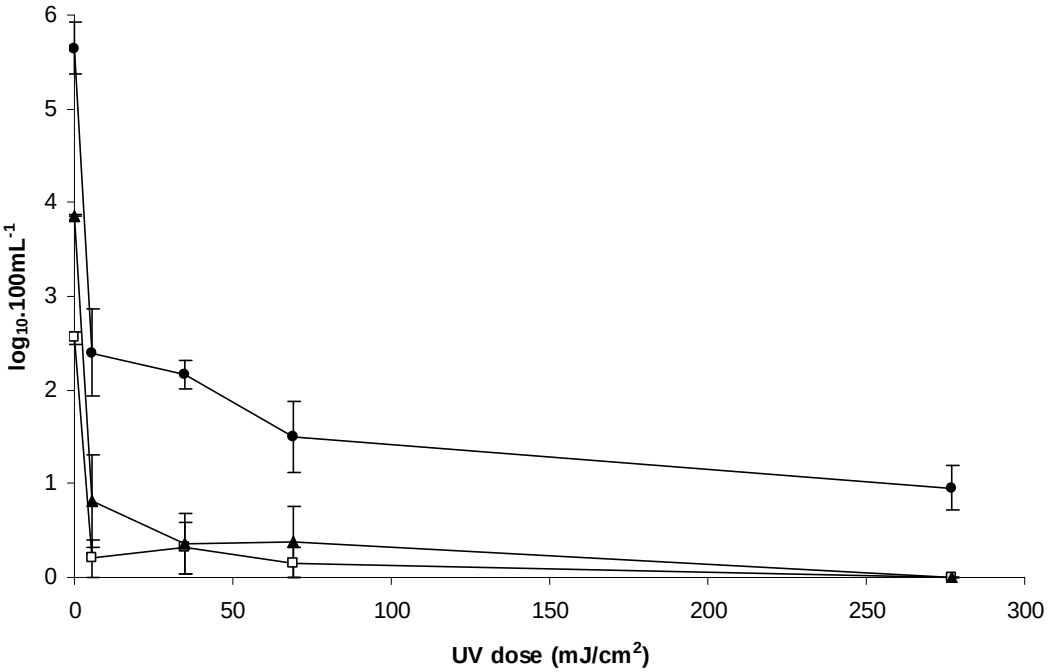

Figure 5.1. *Inactivation of total coliforms (●), E. coli (□), and Enterococci (▲) in grey water by UV light. Data are mean values with standard deviation, n = 3.*

Mamane and Linden (2006) investigated the inactivation of aggregated *Bacillus subtilis* spores by UV light and suggested that greater UV exposure time increased the likelihood of spore exposure to UV light in locations within the aggregate where light pathways exist. Total coliforms were still enumerated in grey water following an extremely high UV dose of 1107 $mJ.cm^{-2}$, at a mean concentration of 0.9±0.4 $log_{10}MPN.100mL^{-1}$ (data not shown), indicating that the grey water contained coliform bacteria deeply embedded within particulate matter and completely shielded from UV light. The data shows that total coliforms are the most conservative of the three bacterial indicators for the disinfection of grey water by UV light due to their higher initial and residual concentrations and are therefore the most suitable for assessment of disinfection performance.

The UV inactivation curve for total coliforms in grey water was compared against inactivation curves for wastewater and treated grey water (reed bed effluent). The wastewater had higher concentrations of BOD and COD compared to the grey water, with a BOD of 42 compared to 20 $mg.L^{-1}$, for example, but had lower TSS and turbidity

values (Table 5.1.). In contrast, the reed bed effluent was low in all water quality parameters compared to the grey water and wastewater, with a BOD of 2 $mg.L^{-1}$. The particle size distributions for the three waters were very different (Figure 5.2.), with distinct peaks in particle size. Volume weighted mean particle sizes for the grey water, wastewater, and reed bed effluent were 505, 56, and 9 µm, respectively (Table 5.1.).

Table 5.1. *Water quality of disinfected waters.*

Water quality parameter	Grey water	Wastewater	HFRB effluent
BOD ($mg.L^{-1}$)	20	42	2
COD ($mg.L^{-1}$)	75	103	17
TSS ($mg.L^{-1}$)	19	12	4
Turbidity (NTU)	18	10	6
pH	7.3	6.7	7.2
UV_{254} transmittance (%, cm^{-1})	47	57	62
Mean particle size (µm)	505	56	9

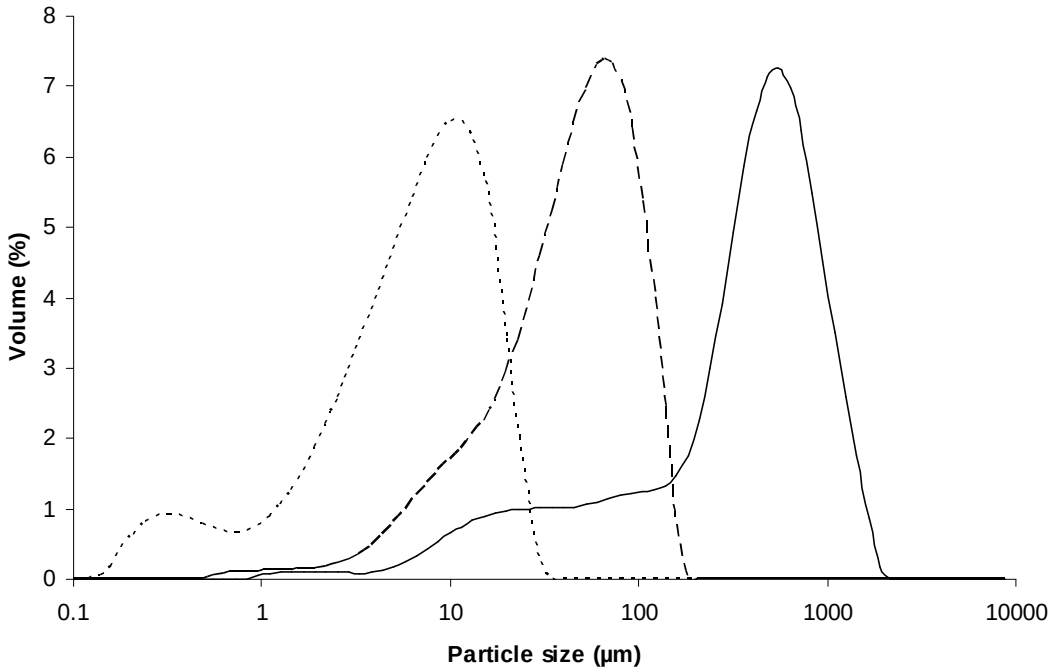

Figure 5.2. *Particle size distributions of grey water (plain line), treated wastewater (long dashes), and HFRB effluent (short dashes).*

Total coliform inactivation by UV light was considerable at low UV doses in all water samples. UV doses of under 10 mJ.cm^{-2}, for example, gave total coliform $\log_{10}$ inactivations of 3.2, 2.1, and 2.4 for the grey water, wastewater, and reed bed effluent samples, respectively (Figure 5.3.). Complete inactivation of total coliforms in the reed bed effluent was achieved at a UV dose of just 11 mJ.cm^{-2}. The smaller mean particle size of the reed bed effluent of 9 µm is not considered large enough to provide shielding to coliform bacteria (Emerick *et al.*, 1999). The grey water and treated wastewater had similar concentrations of total coliforms following high UV doses of over 69 mJ.cm^{-2}. This was particularly interesting because of the differences in water quality between the two waters, particularly the particle size distribution. It could be expected that the larger particle size distribution of grey water would result in greater shielding of coliform bacteria. However, PACs were not extracted from these water samples, following UV disinfection. The data therefore indicates that the grey water and wastewater had similar numbers of particles that were capable of shielding at least one coliform from the UV light at high UV doses.

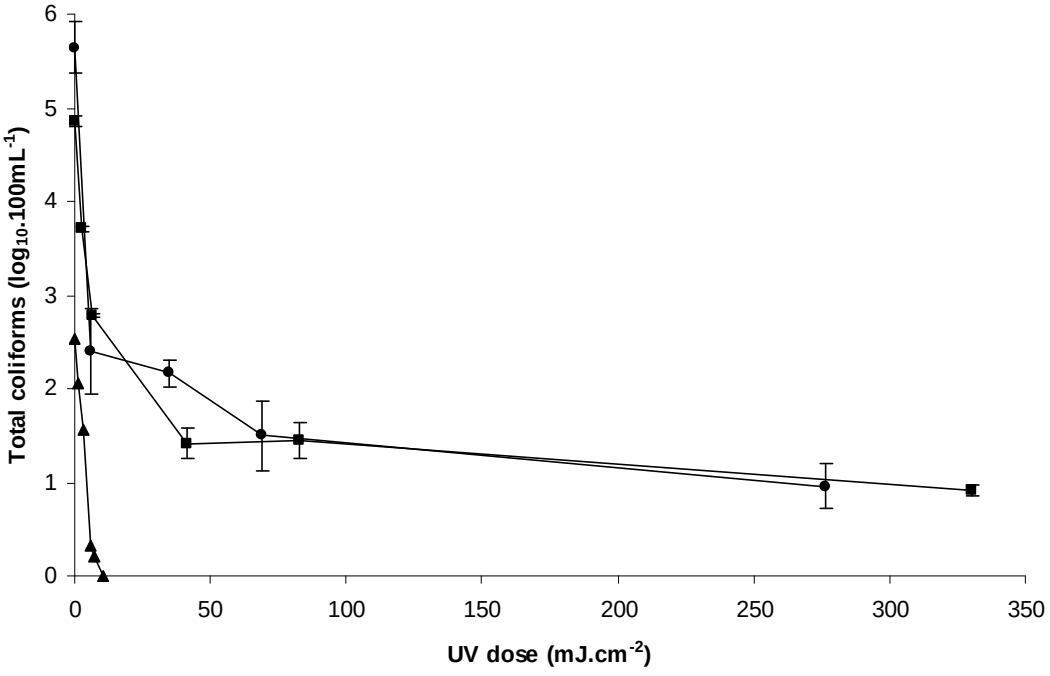

Figure 5.3. *Inactivation of total coliforms by UV light in raw grey water (●), treated wastewater effluent (■), and HFRB effluent (▲). Data are mean values with standard deviation, n = 3.*

Jolis *et al.* (2001) concluded that the efficacy of UV disinfection of filtered wastewater was dependent upon the quantity of particles capable of shielding coliform bacteria from UV light. This is the case when the number of coliforms shielded within each particle is not taken into account. The physical extraction of PACs in wastewater effluent by blending after exposure to UV light revealed total coliform concentrations of between 2 and 340 times greater than non-blended samples (Parker and Darby, 1995). It is hypothesised that the larger particles in grey water are able to shield greater quantities of coliform bacteria from UV disinfection than the smaller particles.

5.3.2. Impact of particle size on UV disinfection

The volume weighted mean particle sizes of the settled grey water fractions ranged from 86 to 607 µm (Table 5.2.). The maximum particle size of a number of the grey water fractions exceeded the detection limit of 2000 µm. In the fraction with the highest mean particle size, however, only 10% of the total volume of particles consisted of particles larger than 1233 µm in size, indicating that the particles over 2000 µm comprised a small proportion of the total particle volume. The fractal dimension of the particles did not change greatly between the fractions, between 1.89 and 1.96, and is indicative of a relatively open particle structure (Jefferson and Jarvis, 2006). Analysis of water quality parameters in relation to particle size of the grey water fractions revealed increases in water quality parameters with increasing particle size. COD, for example, increased from 58 to 132 $mg.L^{-1}$ from the smallest to the largest particle size fraction (Figure 5.4.). Of the measured water quality parameters, TSS was most strongly correlated with particle size, with an R^2 value of 0.95. The UV transmittance remained relatively unchanged in the first four fractions at 73-75% but decreased in the largest particle size fraction to 58% (Table 5.2.).

The survival of total coliforms in UV-irradiated grey water was greater with increasing particle size (Figure 5.5., $R^2 = 0.95$). Total coliform survival climbed from 0.3 to 1.2 $log_{10}MPN.100mL^{-1}$ with an increase in the volume weighted mean particle size from 86 to 607 µm. Blending to extract PACs in the unmodified grey water, prior to UV exposure, revealed that 42% of total coliforms were particle-associated. Exposure to

Table 5.2. *Particle size distribution details and water quality characteristics of grey water particle size fractions.*

Mean particle size (µm)	**86**	**119**	**262**	**470**	**607**
D10 (µm)	10	10	8	73	108
D50 (µm)	55	60	60	377	524
D90 (µm)	206	252	895	1003	1233
Max. particle size (µm)	1660	≥2000	≥2000	≥2000	≥2000
Fractal dimension (D_f)	1.89	1.84	1.89	1.91	1.96
Turbidity (NTU)	10	10	14	16	29
UV_{254} transmittance (%, cm^{-1})	74	75	74	73	58

UV light reduced the overall number of total coliforms in all of the grey water fractions. In the grey water fractions with a mean particle size of 262 µm and above, the proportion of PACs were between 46 and 70% of the total coliform population. Whereas, in the 119 µm particle size fraction, the proportion of PACs was reduced to 23%; and in the 86 µm fraction, blending did not release any further PACs. If it is assumed that all particles in the grey water were of similar porosity, as is indicated by the fractal dimensions of the grey water, the data suggests that large particles, above 262 µm, are more likely to have regions inaccessible to UV light capable of shielding multiple coliforms from inactivation. UV light penetration into a large particle could be expected to reduce towards its centre as light pathways become scarce and UV light is absorbed by the particulate matter. The data indicates that the smaller particle size fractions had a reduced capacity to shield coliform bacteria. The survival of coliform bacteria in the 86 µm fraction after exposure to a high UV dose of 260 $mJ.cm^{-2}$ indicates that the particles were still capable of shielding coliforms from UV light but the lack of an increase in coliform count following blending indicates that the particles in the 86 µm fraction were unable to shield more than one coliform per particle.

The data in this study demonstrates a relationship between particle size and the inactivation of coliforms by UV light. Evidence from the literature supports this finding. Qualls *et al.* (1985) hypothesised that particle size would impact the degree of protection afforded to coliforms from UV light and showed that the survival of coliforms in wastewater effluents exposed to UV light was significantly correlated with the number of particles larger than 40 µm.

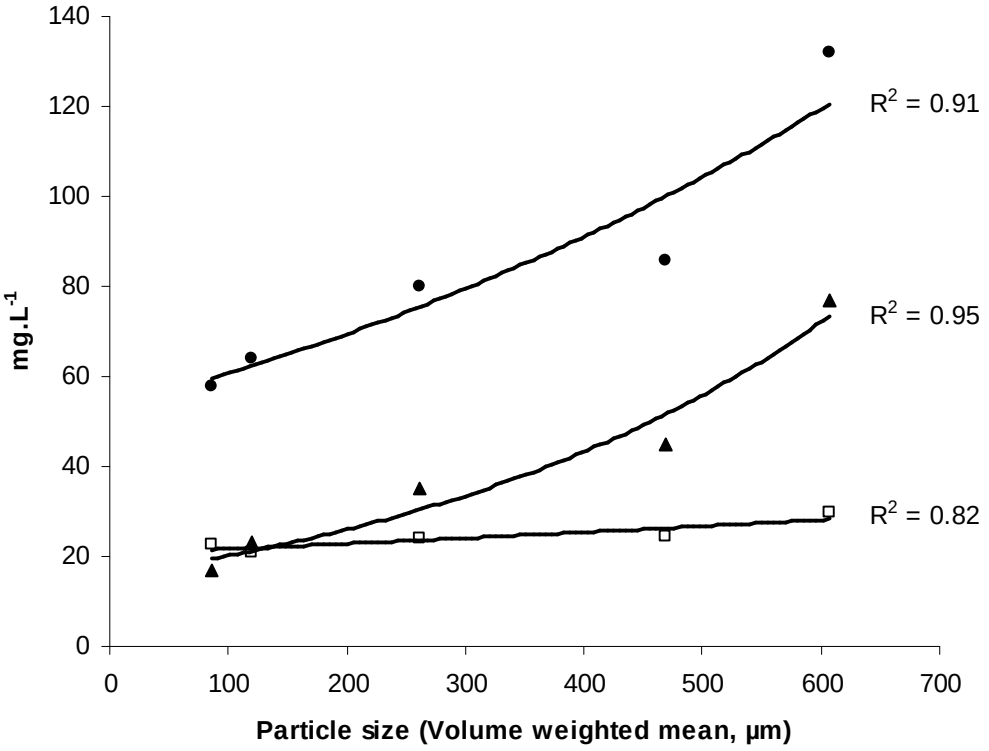

Figure 5.4. *Chemical oxygen demand (COD, ●), total organic carbon (TOC, □) and total suspended solids (TSS, ▲) for particle size fractions of grey water, represented by the volume-weighted mean.*

Madge and Jensen (2006) separated wastewater effluent into different particle size fractions and demonstrated a relationship of decreasing UV disinfection rate with increasing particle size, from <5 µm to >20 µm. At the particle sizes investigated in the current study, protozoa could also be expected to be shielded by particles in the grey water. Viruses are orders of magnitude smaller than coliform bacteria and have been shown to be shielded from UV light by particles of less than 2 µm (Templeton *et al.*, 2005). The large particle sizes in grey water could therefore provide ample protection to embedded viruses.

5.3.3. Impact of organics on UV disinfection

The addition of synthetic grey water supplement to real grey water reduced UV transmittance from 75 to 69 %, cm^{-1} with an increase in TOC from 23 to 197 $mg.L^{-1}$ (Table 5.3.). The UV transmittance was accounted for in the UV dose calculation

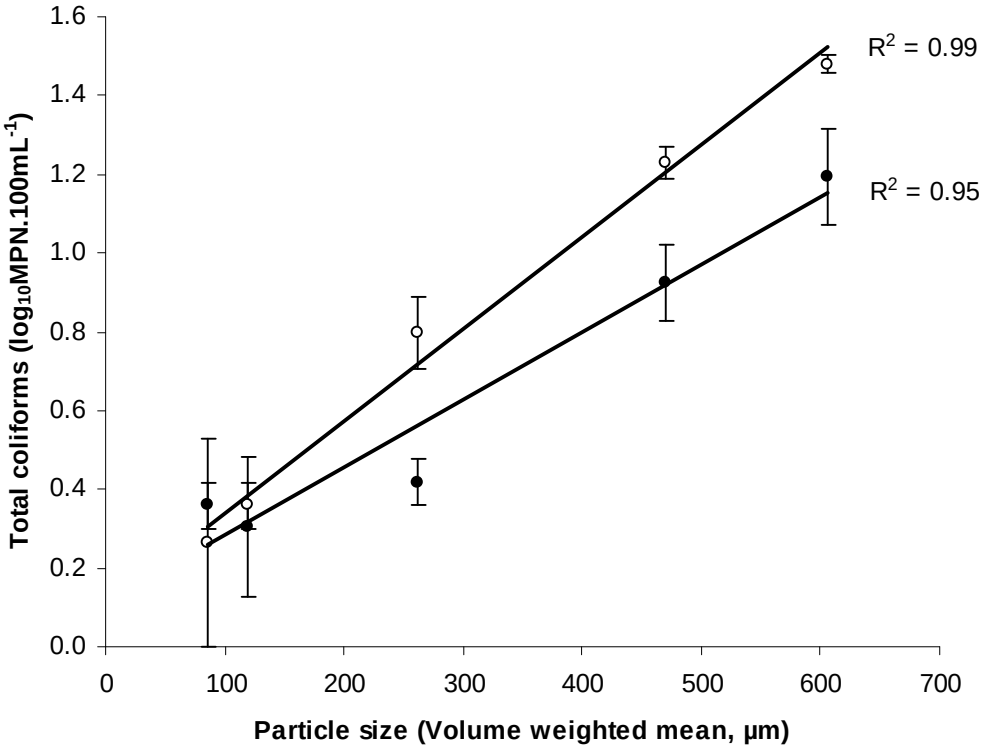

Figure 5.5. *Surviving total coliforms against particle size in manipulated grey water, following UV disinfection at 260 mJ.cm^{-2}). Data shows post-UV blended (○) and non-blended (●) samples. Total coliform concentrations in grey water prior to disinfection were 4.5-5.0 log_{10}MPN.100mL^{-1}. Data are mean values with standard deviation, n = 3.*

allowing application of equivalent UV doses to the different organic strength grey water fractions. Total coliform survival during UV exposure was not affected by the TOC concentration of the grey water. Numbers of total coliforms in the non-blended samples were consistent whereas numbers in the blended samples with extracted PACs were more varied (Figure 5.6). The variation in the numbers of PACs is likely a result of the heterogenous nature of grey water and the resulting uneven distribution of coliform-containing particles between samples. Organics in water are generally considered to limit UV disinfection by reducing UV transmittance through the water and subsequently the UV dose received by the targetted microorganisms. Labas *et al.* (2005), however, reported a protective effect of an organic-rich, particle-free medium on *E. coli* bacteria exposed to UV light. The grey water used in this study already contained significant levels of organics prior to addition of the synthetic supplement and therefore any increased resistance of coliforms due to the presence of additional organics may already

have existed. The data shows that while additional organics in grey water reduce UV transmittance, they do not increase the resistance of coliform bacteria to UV exposure.

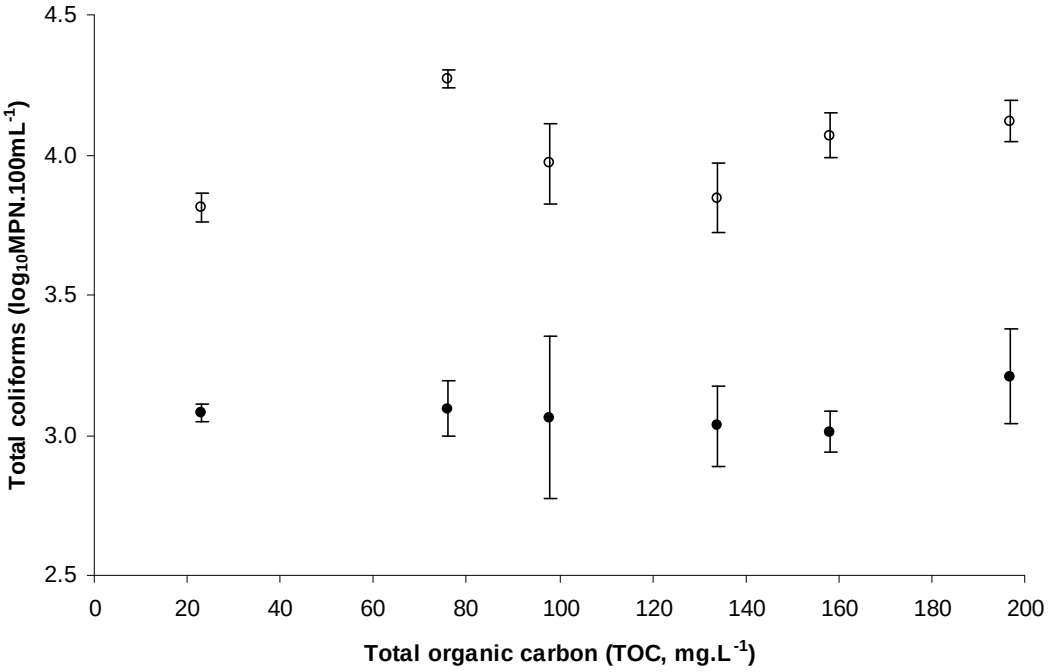

Figure 5.6. *Total coliforms against total organic carbon (TOC) in grey water supplemented with synthetic grey water, following disinfection at a UV dose of 13 mJ.cm^{-2}. Data shows post-UV blended (○) and non-blended (●) grey water. Data are mean values with standard deviation, n = 3. Mean total coliform concentration in grey water prior to disinfection was 6.5 log_{10}MPN.100mL^{-1}.*

5.4. IMPLICATIONS FOR URBAN REUSE

The data presented demonstrate three key points with regards to grey water treatment and disinfection for reuse: (1) the efficacy of UV disinfection of grey water is linked to the size of the particulate material in grey water; (2) the larger particles in grey water shield more coliform bacteria from UV light compared to smaller particles; and (3) additional organics in grey water reduce UV transmittance but do not influence microbial resistance to inactivation by UV light.

UV disinfection of raw, untreated grey water was able to meet the Germany guidelines of 10000 total coliforms per 100mL (equivalent to 4.0 $\log_{10}(y+1).100mL^{-1}$) at the lowest UV dose of 5.8 $mJ.cm^{-2}$. UV doses of up to 1107 $mJ.cm^{-2}$ did not meet the stringent Title 22 criteria for unrestricted urban reuse of 2.2 total coliforms per 100mL. Evidence from the UV disinfection of grey water with different particle size distributions suggests that particle size is a key factor in attaining disinfection to the standards of the Title 22 criteria. Grey water fractions with a mean particle size of ≤119 µm met the Title 22 criteria for total coliforms (equivalent to 0.51 $\log_{10}(y+1).100mL^{-1}$) following a UV dose of 260 $mJ.cm^{-2}$, even with the extraction of PACs (Figure 5.5). Fractions with mean particle sizes above 470 µm did not meet the water reuse criteria at the same UV dose. The 262 µm fraction met the Title 22 criteria without the release of PACs by blending but extraction of PACs increased the total coliform concentration to above 0.51 $\log_{10}.100mL^{-1}$. A UV disinfected grey water could therefore potentially meet the required coliform standards for reuse despite containing over three times the measured number of coliforms as PACs.

Table 5.3. *Water quality characteristics of grey water organic strength fractions.*

TOC ($mg.L^{-1}$)	**23**	**76**	**98**	**134**	**158**	**197**
COD ($mg.L^{-1}$)	44	118	212	318	430	554
TSS ($mg.L^{-1}$)	14	32	23	31	28	30
Turbidity (NTU)	11	11	12	12	12	15
UV_{254} transmittance (%, cm^{-1})	75	73	71	70	70	69

There is limited data on the type, quantity, and frequency of pathogenic microorganisms present in grey water. Pathogens reported in the literature in grey water include *Pseudomonas aeruginosa*, *Salmonella* sp., *Cryptosporidium* sp., and *Giardia* sp. (Casanova *et al.*, 2001; Birks *et al.*, 2004; Birks and Hills, 2007). The tendency for these pathogens to become associated with particles in grey water is not known, however, it would be reasonable to assume that bacterial pathogens would become particle-associated in the same manner as coliform bacteria. The large particle sizes in grey water would also be sufficient to encompass larger protozoan pathogens (Amoah *et al.*, 2005).

Biological treatment of grey water has been recommended due to the high level of organics (Nolde *et al.*, 1999; Jefferson *et al.*, 2004; Pidou *et al.*, 2007). A drawback of biological treatment systems, with respect to UV disinfection, is their propensity to encourage floc development, creating particles with associated coliform bacteria (Emerick *et al.*, 1999). Grey water treated by fluidized-bed reactor and subjected to a UV dose of 25-40 $mJ.cm^{-2}$ contained approximately 2 to 500 total coliforms per 100mL in the final effluent (Nolde *et al.*, 1999). This was sufficient to meet the Germany guidelines for water reuse (<10000 total coliforms.$100mL^{-1}$) but not the California State Title 22 requirements for unrestricted urban reuse.

The removal of particles by filtration through porous media has been shown to improve UV inactivation by removing particles with associated coliform bacteria or viruses (Qualls *et al.*, 1983; Jolis *et al.*, 2001; Templeton *et al.*, 2007). Darby *et al.* (1993) demonstrated that particles were the limiting factor for the disinfection of secondary activated sludge wastewater effluent by UV light to meet stringent water reuse criteria. Tertiary treatment of the wastewater by sand filtration followed by a UV dose of at least $97mJ.cm^{-2}$ consistently met the Title 22 criteria for unrestricted urban reuse. Whereas UV doses of up to 239 $mJ.cm^{-2}$ applied to the unfiltered wastewater effluent did not always meet the the Title 22 criteria. Biological treatment systems that incorporate a filtration step, such as membrane bioreactors (MBR) and porous media reed beds, can provide good organics removal (Dallas and Ho, 2005; Friedler *et al.*, 2006) as well as removal of particles that shield coliforms from UV light. Treatment of grey water through a horizontal flow reed bed allowed complete inactivation of total coliforms in the effluent at a low UV dose of 11 $mJ.cm^{-2}$ (Figure 5.3.). The lack of a tailing region in the inactivation curve for coliforms in the reed bed effluent can be attributed to the removal of the large particles (>10µm) by filtration through the porous reed bed media (Table 5.1.). The mean particle size of the reed bed effluent suggests that the shielding of viruses from UV light would still be of concern. The use of MBRs equipped with ultrafiltration membranes for grey water treatment allows comprehensive removal of particles above a specified membrane pore size. This approach to grey water treatment permits control of particle size in the effluent and removal of particle-associated bacteria, and even viruses, prior to disinfection.

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CHAPTER 6:
ESSENTIAL OILS FOR THE DISINFECTION OF GREY WATER

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6. ESSENTIAL OILS FOR THE DISINFECTION OF GREY WATER

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ABSTRACT

Although the antimicrobial properties of many plant essential oils (EOs) are well known, their application for the disinfection of water has received little attention. In this study, their use as alternative 'natural' disinfectants for grey water reuse was assessed. Toxicity screening of eight EOs and their components highlighted origanum oil (*Thymus capitatus*) and carvacrol as exerting the most antimicrobial activity. Over a 30 minute contact time, origanum EO concentrations of up to 94 $mg.L^{-1}$ had minimal effect on total coliform concentrations in the grey water while a concentration of 468 $mg.L^{-1}$ rendered total coliforms non-detectable in 100mL grey water. Coliform inactivation was found to increase with EO contact time. Organic concentration and particulate size in grey water were shown to reduce the efficacy of disinfection with origanum EO. Origanum EO prevented regrowth of coliform bacteria in reed bed treated grey water for up to 14 days at a concentration of 468 $mg.L^{-1}$, with or without prior disinfection by ultraviolet (UV) light. Based on the disinfection data reported here, the production of sufficient origanum EO for the disinfection of grey water for reuse with toilet flushing, would require approximately 35 times the average land area of a UK household.

6.1. INTRODUCTION

Small-scale, decentralised reuse of water is increasingly viewed as a viable way forward for water conservation in the urban environment (Anderson, 1996; Fane *et al.*, 2002). Small-scale reuse schemes can range from individual households to clusters of homes, such as blocks of flats, or commercial premises like hotels. Water reuse schemes typically involve treatment and disinfection of the source water prior to reuse for applications such as toilet flushing and irrigation.

Grey water can be defined as all wastewater flows exiting a building, with the exception of toilet waste (black water). Depending on the quantity or quality of water required for reuse, grey water is frequently limited to the bathroom streams of hand basin, shower, and bath water, which are less polluted in terms of organics and bacteria (Friedler, 2004; Jefferson *et al.*, 2004). Faecal contamination of grey water and the reported presence of pathogenic microorganisms (Ottoson and Stenström, 2003; Birks *et al.*, 2004) dictates that treatment and disinfection of grey water is important to control the potential health risks emanating from reuse applications. The type of treatment and disinfection employed can vary according to the desired application and regional standards for water reuse. Treatment options for grey water reuse include coarse filtration (March *et al.*, 2004), direct membrane filtration (Ramon *et al.*, 2004), constructed wetland (Dallas and Ho, 2005), rotating biological contactor (Nolde, 1999), and membrane bioreactor (Jefferson *et al.*, 2000).

Chlorine is the leading contender for the disinfection of grey water for reuse. Drawbacks of chlorination include the formation of disinfection by-products that can cause adverse health effects (Morris *et al.*, 1992; Nieuwenhuijsen *et al.*, 2000), and limited efficacy in the presence of particles and organics (thesis chapter 4). Alternative disinfectants that are perceived as 'environmentally friendly' would be an attractive option for operators of small-scale water reuse systems. Such alternatives include ultraviolet (UV) light and plant essential oils (EO).

EOs are aromatic liquids extracted from plant material, typically by steam distillation (Sangwan *et al.*, 2001). EOs and their components have been shown to possess antibacterial (Hammer *et al.*, 1999; Burt, 2004), antiviral (Edris, 2007), antiparasitic (Anthony *et al.*, 2005), antifungal (Soković and van Griensven, 2006), insecticidal (Isman, 2000), and herbicidal (Tworkoski, 2002) properties. Research into the antimicrobial properties of EOs and their components has been largely directed towards their use as food preservatives. EOs and their components have been shown to inhibit bacterial growth in meat, fish, and vegetable products (Burt, 2004). The principal mechanism of bacterial inactivation by EOs is considered to be the disruption of the cell

membrane, causing leakage of cell contents and eventual cell lysis (Burt, 2004). This action of certain EOs can be explained by the presence of phenolic compounds, common in many EOs, such as carvacrol, thymol, and eugenol, which are known to cause such disturbance of the cell membrane (Sikkema *et al.*, 1995).

The effective use of EOs as inhibitors of microbial growth in foods points to their potential as residual regrowth inhibitors in reused water. This study investigates the disinfection potential of EOs for grey water reuse, including the impact of organics and particles, and their application as residual regrowth inhibitors in reed bed treated grey water following UV disinfection.

6.2. MATERIALS AND METHODS

6.2.1. Water sample collection

Grey water was collected from bathroom sinks, baths and showers of 18 specially plumbed student flats on Cranfield University campus. The grey water was collected by gravity in an underground sump and then pumped into a continuously circulating holding tank (700L capacity). The grey water was supplemented with a 10% v/v shampoo (Tesco Value) in tap water to create a 'high strength' grey water. The supplementary solution and the real grey water were pumped at fixed intervals into a second holding tank from which the mixture was pumped to the VFRB for treatment. The supplemented grey water is referred to as 'high strength'. A self-contained vertical-flow reed bed (VFRB) (RIB, Oceans-ESU, Bradford, UK) was fed high strength grey water at a rate of 480L.d^{-1} in 10 batches of 48L via a centrifugal pump on a timer. The VFRB was of 6m^2 surface area and 0.7m depth, and consisted of a sand/soil/compost mixed media planted with *Phragmites australis*. The hydraulic retention time per batch was determined by lithium tracer study as 2h. All samples were collected in sterile polypropylene containers and either used immediately or stored at 5±1°C and used within 2 hours.

6.2.2. Water quality analysis

Biological and chemical oxygen demand (BOD, COD), total organic carbon (TOC), and total suspended solids (TSS) were calculated according to standard methods (APHA, 1998). BOD was determined by the 5 day incubation method, while total COD and TOC were analysed using the Merck spectroquant method. Measurement of particle size distributions was carried out using a Mastersizer 2000 (Malvern, UK). For each measurement of particle size distribution five repetitions were carried out using a measurement and background measurement time of 25s and a refractive index of 1.52. The manufacturer specifies a minimum and maximum detection limit of 0.2 µm and 2000 µm, respectively. The volume weighted mean D[4,3] was used to represent the particle size distribution. D10, D50, and D90 values refer to the diameter below which lie a percentage of the total volume of all particles in the sample. Total coliforms were enumerated by most probable number (MPN) method using IDEXX Colisure and Quanti-Tray (Maine, USA), according to standard methods (APHA, 1998).

6.2.3. Grey water manipulation

Grey water was manipulated to assess the impacts of particle size and organic concentration on the efficacy of disinfection with essential oils. The methodology has been previously reported elsewhere (thesis chapter 4) and are described briefly here. Grey water fractions of varying particle size distributions were created by a settling process, using settling times of between 10 to 60 minutes to provide grey water with a range of particle size distributions. Grey water was manipulated in terms of organic content by the addition of a synthetic grey water concentrate, consisting of shampoo, shower gel, and vegetable oil (20:20:1) in deionised water, to create fractions of different organic concentration, as measured by Merck spectroquant TOC cell test. Particle size distribution, TSS, TOC, and COD were measured for all fractions. Initial origanum oil concentrations of 94, 281, and 468 $mg.L^{-1}$ were applied to the particle size fractions and 281 $mg.L^{-1}$ to the organic strength fractions, for a set contact time of 30 min. Total coliforms were enumerated following disinfection.

6.2.4. Essential oils

Essential oils and components were obtained from Sigma-Aldrich (Dorset, UK) and used as received. Volumes were converted to mass using specific gravity data for the tested compounds (Table 6.1.) although the original volumes have been included in parentheses in the results to aid comparison with literature data. Origanum oil, which was used in the majority of experiments, had been extracted by steam distillation from *Thymus capitatus* L. (Labiatae) Hoffmanns & Link (common name: Spanish oregano; synonyms: *Coridothymus capitatus* or *Thymus capitatis*) and contained 65.4% phenols by volume.

Table 6.1. *Biological source and specific gravity of essential oils.*

Essential oil	Biological source	Specific gravity ($g.cm^{-3}$)
Origanum Oil	*Thymus capitatus*	0.936
Thyme Oil	*Thymus vulgaris*	0.916
Tea Tree Oil	*Melaleuca alternifolia*	0.898
Lemongrass Oil	*Cymbopogon citratus*	0.900
Rosemary Oil	*Rosemarinus officinalis*	0.908
Clove Bud Oil	*Eugenia* spp.	1.040
Coriander Oil	*Coriandrum sativum*	0.868
Peppermint Oil	*Mentha piperita*	0.900

6.2.5. Toxicity screening of essential oils

The relative toxicity of the essential oils and active ingredients was assessed using the Microtox Acute Toxicity Test (81.9% basic test). In the test, luminescent bacteria, *Vibrio fischeri*, are exposed to a range of dilutions of the tested compound and the impact on light output is measured. The test compares the light output of the bacteria after exposure to a test compound with the light output of a control, containing no test compound. A reduction in light output in the bacteria exposed to the test compound compared to the control is attributed to a toxic effect of the test compound on the bacteria. By plotting a range of concentrations of the test compound against the measured decrease in light output, an EC_{50} (Effect Concentration 50%) value can be derived that describes the concentration of the test compound at which a 50% reduction

in light output is observed. The lower the EC_{50}, the more toxic the compound. The Microtox method allows screening of multiple test compounds for their toxic effect on bacteria, which provides an indication of the effectiveness of compounds for bacterial inactivation and thus disinfection.

Microtox testing was carried out in accordance with the manufacturer's instructions and measurements were taken after 5, 15, and 30 minutes. Essential oils and active ingredients were diluted in autoclaved, sterile 0.1% agar solution prior to toxicity testing. The use of agar as a chemically and microbially inert stabiliser has been shown to be an effective way of creating homogenous suspensions of essential oils while maintaining their antimicrobial properties (Mann and Markham, 1998; Burt and Reinders, 2003). Thymol was first dissolved in 1:1 ethanol/distilled water. The 0.1% agar solution was verified as exerting no toxicity according to the Microtox test. Chlorine, as sodium hypochlorite, was included in the toxicity screening for comparison. The concentration of chlorine as Cl_2 in the sodium hypochlorite was measured by titration using standard methods (APHA, 1998)

6.2.6. Essential oil disinfection

All water samples and solutions were equilibrated to room temperature (18 ± 1°C). A volume of origanum EO was added directly to a water sample (≤0.5% of total volume) in a pyrex bottle to provide the desired concentration. The sample was shaken vigorously for 10 seconds to ensure dispersal of the essential oil throughout the sample and kept in darkness at 18 ± 1°C. After the designated contact time, samples were shaken vigorously for 10 seconds and total coliforms were enumerated.

Unlike other methods of disinfection, such as UV light or chlorine, EOs remain in the water after the desired contact time and cannot be neutralised in the same way as chlorine. The EO will also remain in the water during the enumeration procedure, potentially continuing to exert antimicrobial activity and preventing growth of total coliforms for enumeration. In order to obtain the most accurate assessment of essential oil disinfection performance possible, the following steps were taken: (1) tests were

staggered so that after the desired time of exposure to EO, the sample was immediately processed for enumeration of total coliforms, and (2) total coliforms and *Escherichia coli* were enumerated from a full range of ten-fold dilutions (thereby reducing the EO concentration with each dilution) and the dilution with the highest bacterial count was recorded.

6.2.7. UV disinfection

UV inactivation experiments and collimated beam operation were carried out as described by Bolton and Linden (2003). Water samples were allowed to equilibrate to room temperature (18 ± 1°C) prior to their use in the inactivation experiments. 300mL aliquots were added to a 20cm diameter petri dish, giving a 1cm sample depth, and were magnetically stirred by a sterile 2cm stirrer bar at 100rpm for 10 seconds prior to, and during, UV exposure. Samples were exposed to 254nm UV-C irradiation from a collimated beam device (Wedeco AG, Germany) consisting of four low pressure UV lamps and a pneumatically controlled shutter. The applied UV fluence rate was determined by uridine actinometry (DVGW, 1997) as 2.08mW/cm^2 and the UV dose (or fluence) was controlled by exposure time. UV exposure time was adjusted to correct for the UV transmittance and depth of the sample. UV_{254} absorbance of samples was measured through a 1cm quartz cell in a UV-Vis spectrophotometer. The spectrophotometer was not equipped with an integrating sphere. This could have resulted in an overestimation of UV absorbance, as light reflected off particles and not passing directly through the sample impacts the absorbance measurement (Christensen and Linden, 2003). Following UV exposure, samples were transferred to sterile bottles and used immediately or stored at 5 ± 1°C for a maximum of 2 hours prior to total coliform enumeration.

6.2.8. Regrowth experiments with UV and essential oils

VFRB effluent and UV-exposed VFRB effluent were placed into 2L polypropylene bottles. Initial origanum EO concentrations of 0, 9, 47, 94, and 468 mg.L^{-1} were applied and each sample was shaken vigorously for 10 seconds and stored in the dark at 18 ±

1°C. Total coliforms were enumerated periodically for up to 14 days from each sample, which was mixed prior to the enumeration.

6.3. RESULTS AND DISCUSSION

6.3.1. Toxicity screening

EC_{50} concentrations ranged from 0.23 to 21.50 $mg.L^{-1}$ across the different EOs and components tested (Table 6.2.). Origanum and thyme oils exerted the most toxicity of all the essential oils, with EC_{50} (5 min) concentrations of 0.27±0.02 $mg.L^{-1}$ (0.29 $\mu l.L^{-1}$) and 1.07±0.12 $mg.L^{-1}$ (1.16 $\mu l.L^{-1}$), respectively. The EC_{50} concentrations are comparable with literature data, collated by Burt (2004), showing minimum inhibitory concentrations (MICs) for origanum and thyme oils of 0.5-1.3 $\mu l.L^{-1}$ against *E. coli* and 0.2-2.5 $\mu l.L^{-1}$ against *Staphylococcus aureus*. The toxicity of origanum oil was similar to that of chlorine (hypochlorite), which had an EC_{50} concentration of 0.28±0.04 $mg.L^{-1}$. Peppermint oil was the least toxic EO tested with an EC_{50} (5 min) concentration of 16.75±4.36 $mg.L^{-1}$ (18.61 $\mu l.L^{-1}$).

Key EO components were separately tested revealing that carvacrol, γ-terpinene, and thymol were the most toxic with EC_{50} (5min) values of 0.23±0.01 (0.23 $\mu l.L^{-1}$), 1.23±0.03 $mg.L^{-1}$ (1.45 $\mu l.L^{-1}$), and 2.86±0.06 $mg.L^{-1}$ (3.12 $\mu l.L^{-1}$), respectively (Table 6.2.). Carvacrol and thymol are key components of origanum and thyme oils, and γ-terpinene is a biosynthetic precursor to these compounds. The toxicity of γ-terpinene was surprising because it is not considered to exert antimicrobial activity (Sivropoulou *et al.*, 1996) as it is unable to penetrate the bacterial outer membrane (Mann *et al.*, 2000). The data indicates that *Vibrio fischeri* bacteria are susceptible to γ-terpinene. For the majority of compounds tested an increase in EC_{50} was observed at 30 min compared to 5min contact time. There are two possible explanations for this: (1) a reduction in effective concentration due to the loss of a volatile compound to the atmosphere or a reaction with bacterial cells; or (2) the bacteria can show acclimatisation to EO exposure (Dimitrijević *et al.*, 2007).

Table 6.2. *EC50 values for essential oils, components, and chlorine, for 5 and 30 minute contact times. Data are mean values with standard deviation. For essential oils and chlorine, n =3; for components, n = 2.*

		EC50 ($mg.L^{-1}$)	
		5min	30 min
Essential oils	Origanum Oil	0.27 ± 0.02	0.33 ± 0.06
	Thyme Oil	1.07 ± 0.12	1.21 ± 0.19
	Tea Tree Oil	3.29 ± 0.37	3.86 ± 0.61
	Lemongrass Oil	4.03 ± 0.07	4.45 ± 0.11
	Rosemary Oil	4.27 ± 0.33	2.25 ± 0.15
	Clove Bud Oil	5.04 ± 0.33	5.18 ± 0.24
	Coriander Oil	5.86 ± 1.26	5.38 ± 1.45
	Peppermint Oil	16.75 ± 4.36	21.50 ± 4.92
Components	Carvacrol	0.23 ± 0.01	0.26 ± 0.03
	γ-Terpinene	1.23 ± 0.03	2.51 ± 0.15
	Thymol	2.86 ± 0.06	2.14 ± 0.09
	Geraniol	3.20 ± 0.02	3.32 ± 0.28
	Eugenol	4.08 ± 0.48	4.97 ± 0.56
	Citronellal	6.99 ± 0.07	6.43 ± 0.07
	Linalool	8.93 ± 0.09	10.13 ± 0.13
	Limonene	12.72 ± 0.25	16.20 ± 0.38
Chlorine (Cl_2 as hypochlorite)		0.28 ± 0.04	0.40 ± 0.04

The Microtox toxicity test was used as a screening method to select EOs suitable for the purposes of disinfection. The test appeared to be a good indicator of disinfecting potential as the findings are supported by literature data, identifying origanum and thyme EOs as some of the most powerful antimicrobials compared to other EOs (Hammer *et al.*, 1999; Burt and Reinders, 2003; Soković and van Griensven, 2006). Also, Peñalver *et al.* (2005) reported that the antimicrobial activity of EOs towards enteric bacteria was greater in those oils with higher concentrations of the phenolic components carvacrol and thymol. Indeed, Lambert *et al.* (2001) demonstrated that the inhibitory effect of origanum oil on *Pseudomonas aeruginosa* and *Staph. aureus* bacteria was principally due to the additive effect of its key components, carvacrol and thymol. From the toxicity data, and supporting literature data, origanum oil from *Thymus capitatus* was selected as the strongest candidate for further study. The origanum EO of *Thymus capitatus* contains a combination of carvacrol and thymol that typically constitutes between 54 and 83% of the reported compounds in the oil (Karpouhtsis *et al.*, 1998; Fleisher and Fleisher, 2002; Goren *et al.*, 2003).

6.3.2. Inactivation curves

The inactivation of total coliforms or *E. coli* in grey water was not observed at origanum EO concentrations of up to 94 $mg.L^{-1}$ over a 30 minute contact time (Figure 6.1.). This lag phase in the disinfection curve is typically observed for the chlorine disinfection of wastewater, where it indicates the reaction of the disinfectant with compounds in the water (Warton *et al.*, 2006). At EO concentrations of 187 $mg.L^{-1}$ and above, bacterial concentrations reduced with increasing EO concentration. In contrast to the chlorine disinfection of grey water (thesis chapter 4); no tailing was observed at higher EO concentrations. *E. coli* and total coliforms were undetected following EO concentrations of 374 and 468 $mg.L^{-1}$, respectively.

Total coliform inactivation in grey water was observed to increase with increasing EO contact time (Figure 6.2.). An EO concentration of 281 $mg.L^{-1}$ was selected as it impacted coliform concentrations after 30 minutes contact time. Most inactivation of total coliforms occurred within the first five minutes of exposure, with a log_{10} removal of 1.9, increasing to just 2.4 after 15 minutes contact time. Complete inactivation of total coliforms was achieved at a contact time of 60 minutes. A similar effect of increasing inactivation with contact time was reported by Sivropoulou *et al.* (1996) who showed complete inactivation of *Staph. aureus* bacteria exposed to 250 $\mu l.L^{-1}$ origanum (*Origanum vulgare*) EO after 60 minutes.

The concentration of origanum oil required for a bactericidal effect on total coliforms in grey water was 187 $mg.L^{-1}$, much higher than the EC_{50} (30 min) concentration of 0.33 ± 0.06 $mg.L^{-1}$ against *Vibrio fischeri*. Although the single-microorganism Microtox test is considered a particularly sensitive test for toxicity compared to diverse microbial populations such as those in wastewater (Ricco *et al.*, 2004), a study by Goren *et al.* (2003) reported the inhibition of growth of the coliform bacteria *E. coli* and *Klebsiella pneumoniae* following exposure to origanum EO (*Thymus capitatus*) at concentrations of just 1.1 to 4.4 $mg.L^{-1}$, respectively. This data shows that bacteria in grey water are much more resistant to disinfection by origanum EO than those in pure culture and indicates that constituents in the grey water are responsible for limiting the efficacy of

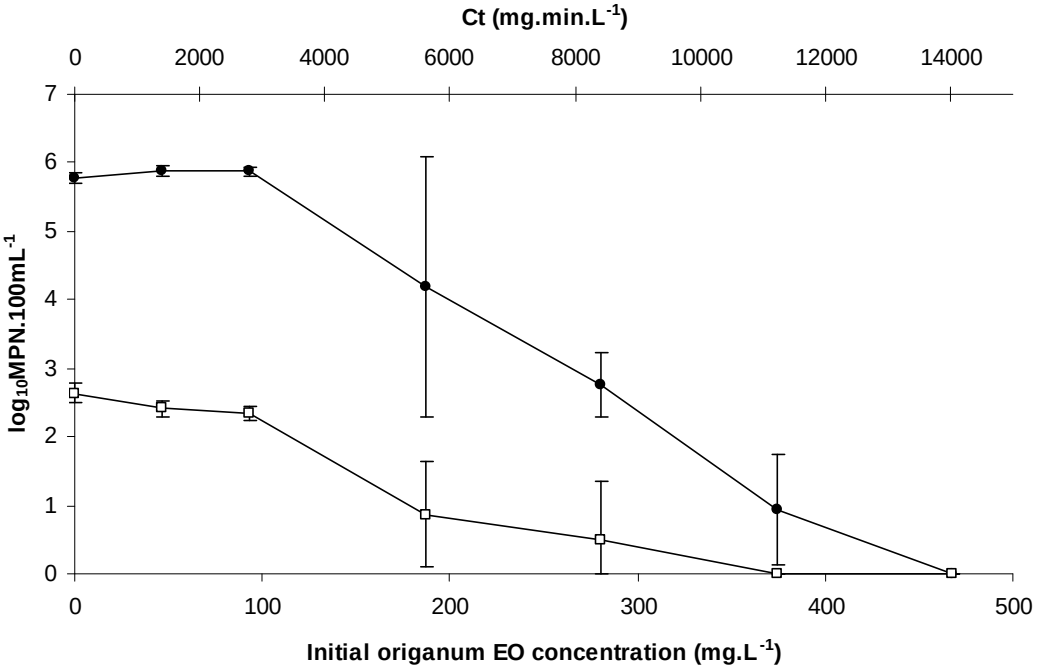

Figure 6.1. *Inactivation of total coliforms (●) and E. coli (□) in grey water by Origanum EO over a 30 min contact time. Data are mean values with standard deviation, n = 3.*

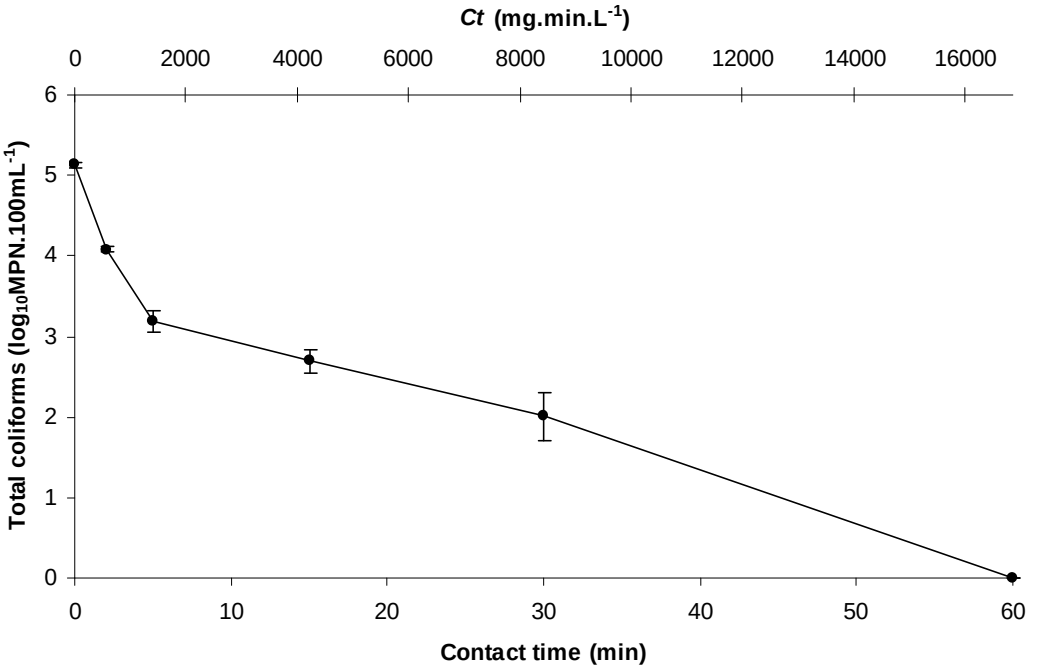

Figure 6.2. *Inactivation of total coliform bacteria in grey water by origanum EO (281 mg.L^{-1}) with increasing contact time. Data are mean values with standard deviation, n = 3.*

disinfection with origanum EO. The impact of water quality on the disinfection efficacy of origanum EO was further investigated.

6.3.3. Impact of organics on EO disinfection

A clear relationship was observed between organics in grey water and the efficacy of EO disinfection. Increasing TOC was linked to a reduction in the inactivation of total coliforms (Figure 6.3., $R^2 = 0.98$). To illustrate, for a TOC of 76 $mg.L^{-1}$, the surviving total coliform concentration was 2.1 $log_{10}MPN.100mL^{-1}$, whereas for a TOC of 142 $mg.L^{-1}$ total coliform survival increased to 4.1 $log_{10}MPN.100mL^{-1}$.

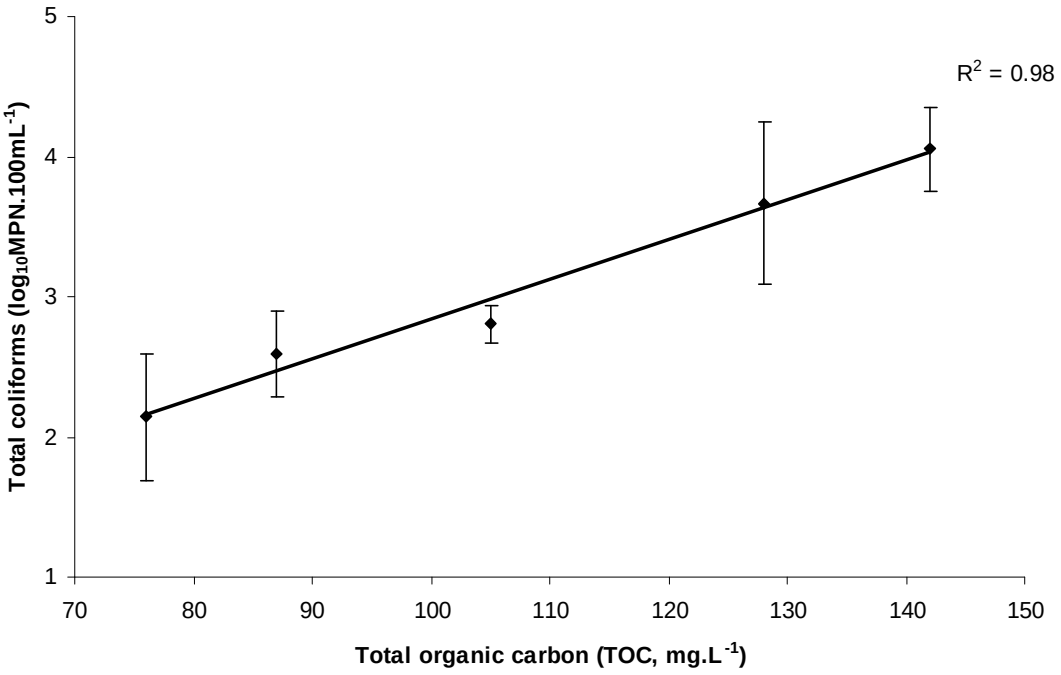

Figure 6.3. *Total coliform survival against total organic carbon (TOC) in grey water supplemented with synthetic grey water, following disinfection with origanum EO at a concentration of 281 $mg.L^{-1}$. Data are mean values with standard deviation, n = 3. Mean total coliform concentration in the grey water prior to disinfection was 4.5 $log_{10}MPN.100mL^{-1}$.*

The interactions between organic matter and essential oils are not well understood. Fats and proteins in foods have been shown to limit the antibacterial activity of EOs (Juven *et al.*, 1994; Singh *et al.*, 2003), with a greater concentration of EO typically needed to achieve a similar level of microbial inhibition in foodstuffs compared to laboratory

assays (Shelaf *et al.*, 1984; Burt, 2004). A key mechanism of bacterial inactivation of EOs is the disruption of the cell membrane (Rhayour *et al.*, 2003; Burt, 2004). Carvacrol and thymol, key components of origanum EO, trigger disintegration of the bacterial cell membrane and increase cell permeability (Helander *et al.*, 1998; Lambert *et al.*, 2001). It has been suggested that organic matter binds to the phenolic components of EOs, reducing the EO concentration available to react with the bacterial cell membrane (Juven *et al.*, 1994). Organic matter therefore appears to exert a 'demand' for EO as it does with chlorine, reducing the concentration available for disinfection.

6.3.4. Impact of particle size on EO disinfection

The effect of particle size on disinfection varied with the EO concentration (Figure 6.4.). At the lowest origanum EO concentration of 94 $mg.L^{-1}$, consistent total coliform log_{10} reductions of between 0.7 and 1.2 were observed, regardless of the particle size fraction. Whereas, at 281 $mg.L^{-1}$, similar log_{10} coliform removals of between 0.9 and 1.3 were achieved in the particle size fractions of 440 µm and above. However, in the smaller particle size fractions, coliform survival was markedly lower, with a log_{10} removal of 2.8 in the 268 µm fraction, for example, while coliforms were not detected in the particle size fractions of 110 µm and below. Complete inactivation of coliform bacteria was achieved with an origanum EO concentration of 468 $mg.L^{-1}$ in the particle size fractions up to 465 µm. Whereas, in the larger particle size fractions, above 637 µm, coliforms were observed to survive disinfection, despite log_{10} removals of 1.9 to 2.5.

The data indicates that the particle size distribution of grey water impacts the efficacy of disinfection with origanum EO. The largest particle size fractions, with mean particle sizes of 637 µm and above, increased the resistance of coliform bacteria to inactivation, even at the highest EO concentration of 468 $mg.L^{-1}$. The particle size of the settled grey water fractions was closely linked to the water quality parameters of COD, TOC, and TSS, with R^2 values of 0.92 to 0.98 (Table 6.3.). The increase in TOC concentration was low, just 16 $mg.L^{-1}$, indicating that the contribution of organics to the increased resistance of coliform bacteria in the larger particle size fractions was minimal.

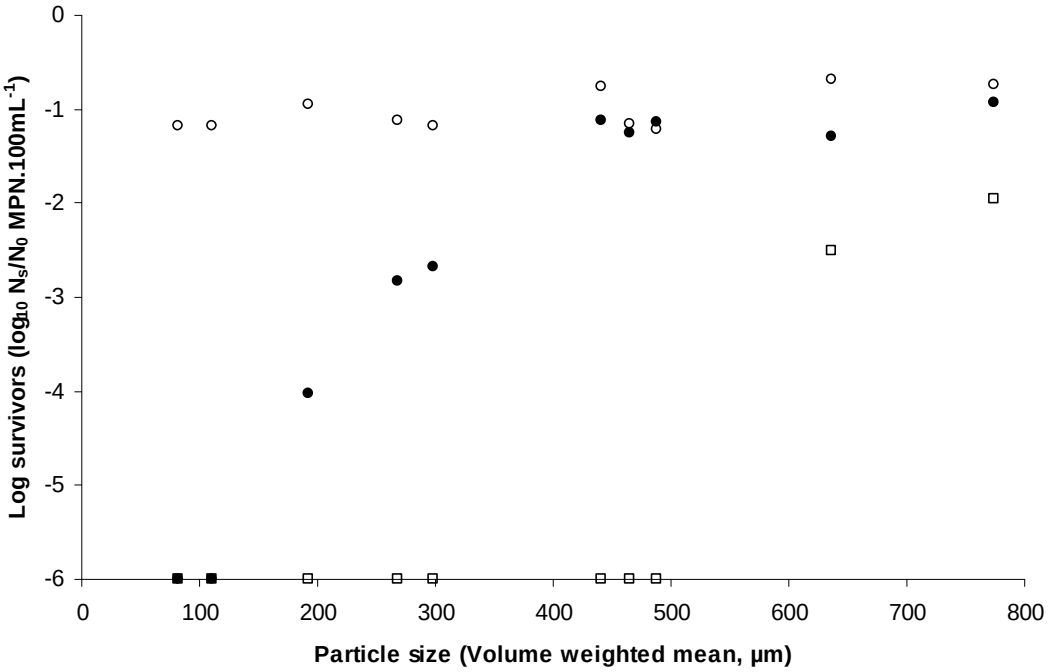

Figure 6.4. *Proportion of surviving total coliforms against particle size in manipulated grey water, following application of initial origanum EO concentrations of 94 (○), 281 (●), and 468 (□) mg.L^{-1}. Cases of zero coliform survival have a value of -6.*

The increased resistance of microorganisms to disinfection in particle-containing waters has been reported for a range of disinfection systems, including chlorine (Berman *et al.*, 1988), chlorine dioxide (Narkis *et al.*, 1995), ozone (Dietrich *et al.*, 2007), and ultraviolet light (Qualls *et al.*, 1983). Particles of around 10 µm are capable of shielding coliform bacteria from chlorine and UV disinfection (Ridgeway and Olson. 1982; Emerick *et al.*, 1999), while larger particles have been shown to shield significant numbers of coliform bacteria from UV disinfection (Parker and Darby, 1995). The proportion of particle-associated coliforms shielded from EO disinfection could not be measured because there is currently no method to neutralise the EO, meaning coliforms extracted from particles and dispersed by physical or chemical extraction methods would be subsequently exposed to the EO disinfectant. Additional coliform bacteria embedded in particles may therefore have persisted and were not enumerated in this study.

Table 6.3. *Particle size distribution details and water quality characteristics of grey water fractions.*

Mean particle size (µm)	D10 (µm)	D50 (µm)	D90 (µm)	Max. particle size (µm)	COD ($mg.L^{-1}$)	TOC ($mg.L^{-1}$)	TSS ($mg.L^{-1}$)
81	10	47	195	631	62	20	19
110	11	75	267	832	68	21	22
193	12	97	416	1445	70	22	27
268	19	180	507	1660	74	26	33
298	21	235	689	≥2000	73	25	35
440	77	383	882	1905	82	28	54
465	72	377	988	≥2000	88	27	46
488	79	411	1002	≥2000	92	28	58
637	104	561	1291	≥2000	122	32	76
774	168	733	1424	≥2000	162	36	137

6.3.5. Essential oils as regrowth inhibitors

Treatment of high strength grey water by the VFRB greatly reduced all water quality parameters (Table 6.4.). For example, COD and TSS were reduced from 412 and 25, to 24 and <1 $mg.L^{-1}$, respectively. Treatment also reduced the mean particle size of the grey water; from 162 to 36 µm. Large numbers of total coliforms, 5.9 $log_{10}MPN.100mL^{-1}$, remained in the VFRB effluent. UV disinfection provided complete inactivation of coliforms in the VFRB effluent following a UV dose of 52 $mJ.cm^{-2}$ (data not shown). However, for subsequent experiments, a UV dose of 26 $mJ.cm^{-2}$ was selected to leave a low, residual concentration of coliform bacteria, allowing assessment of regrowth potential in the VFRB effluent.

Table 6.4. *Water quality of high strength grey water and treated VFRB effluent.*

Water quality parameter	Grey water (high strength)	VFRB effluent
COD ($mg.L^{-1}$)	412	24
TOC ($mg.L^{-1}$)	82	61
TSS ($mg.L^{-1}$)	25	<1
Turbidity (NTU)	34	2
UVT (%, cm^{-1})	64	62
Mean particle size (µm)	162	36
pH	7.0	6.9
Total coliforms ($log_{10}MPN.100mL^{-1}$)	7.3	5.9

In the VFRB effluent not exposed to UV or EO, total coliform concentration remained relatively constant over 7 days, between 5.5 and 6.2 $log_{10}MPN.100mL^{-1}$ (Figure 6.5.). EO concentrations of up to 94 $mg.L^{-1}$ had little effect on the coliform numbers, with values ranging between 5.6 and 6.5 $log_{10}MPN.100mL^{-1}$ for up to 7 days. At the highest EO concentration of 468 $mg.L^{-1}$, coliforms were undetected after 1 hour and regrowth was not observed for up to 14 days. UV exposure reduced the concentration of coliforms in the VFRB effluent to 1.6 $log_{10}MPN.100mL^{-1}$. The addition of 9 $mg.L^{-1}$ EO caused no further reduction in the coliform concentration (Figure 6.6.). At EO concentrations of 47 and 94 $mg.L^{-1}$, coliforms were not detected for up to 4 and 8 hours, respectively. After 3 days, however, the coliform concentrations were back to between 1.4 and 1.9 $log_{10}MPN.100mL^{-1}$. The addition of 468 $mg.L^{-1}$ EO rendered coliforms undetectable after 0.5 hours and for up to 14 days.

The data show that a high concentration, 468 $mg.L^{-1}$, of origanum EO was effective for inhibiting the regrowth of coliform bacteria in reed bed treated grey water, with or without prior disinfection by UV. Lower origanum EO concentrations limited coliform regrowth in UV disinfected effluent but only for up to 8 hours. The use of UV disinfection coupled with origanum EO therefore provided little additional benefit compared to origanum EO alone. Disinfection with origanum EO was limited by organics, which rendered the disinfectant ineffective against coliform bacteria in the grey water or treated reed bed effluent at concentrations below 94 $mg.L^{-1}$. Grey water treated to a higher standard, such as by membrane bioreactor (Jefferson *et al.*, 2001) would likely require lower concentrations of origanum EO for effective inhibition of regrowth.

6.3.6. Practical implications for urban water reuse

The disinfection of grey water and reed bed treated grey water by origanum EO was most effective at concentrations of 468 $mg.L^{-1}$ (500 $\mu L.L^{-1}$). This concentration can be considered high, however, when taking into account that EO yield from plant material is typically low, less than 2% (v/w) for *Thymus capitatus* (Hedhili *et al.*, 2002).

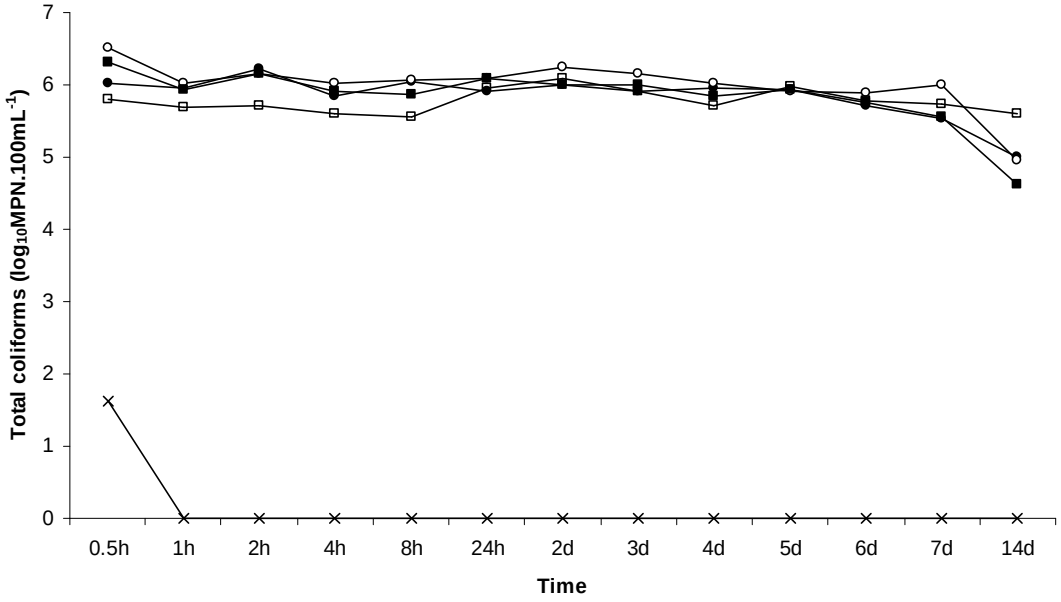

Figure 6.5. *Regrowth of total coliforms over time in VFRB effluent following addition of origanum EO concentrations of 0 (●), 9 (○), 47 (■), 94 (□), and 468 (×) mg.L^{-1}.*

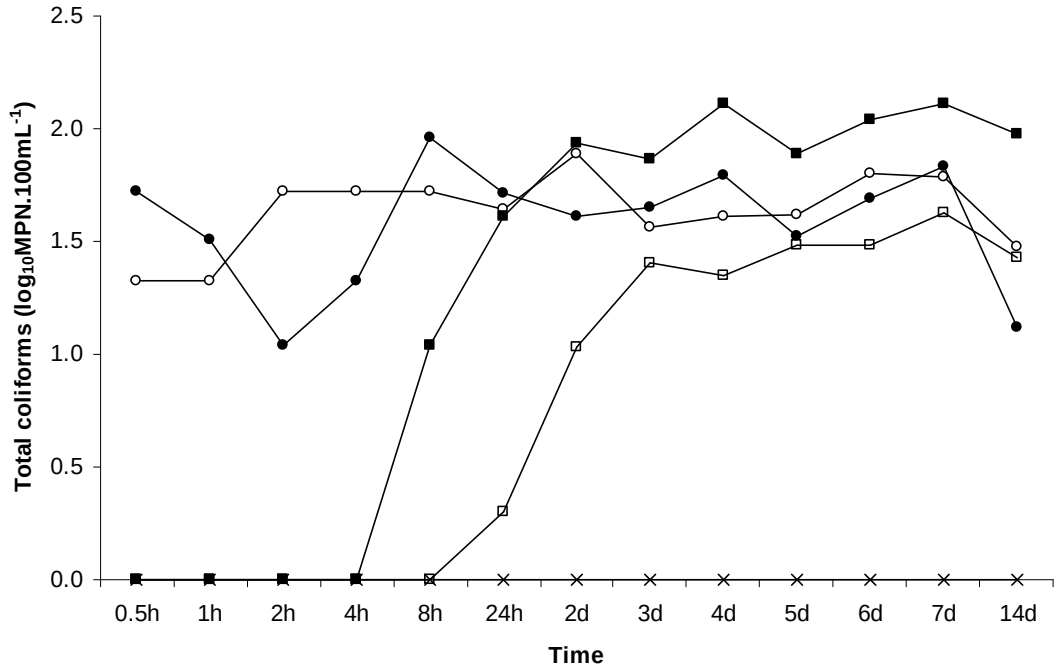

Figure 6.6. *Regrowth of total coliforms over time in VFRB effluent following a UV dose of 26 mJ.cm^{-2} and addition of origanum EO concentrations of 0 (●), 9 (○), 47 (■), 94 (□), and 468 (×) mg.L^{-1}.*

In an attempt to quantify the feasibility and environmental impact of using EOs for disinfection, the land area required to produce sufficient origanum EO to disinfect grey water for reuse with toilet flushing, was estimated. A number of assumptions were made based on literature data. An EO yield of 50L.ha^{-1} was assumed, based on optimum reported yields for another *Thymus* sp. (Baranauskiene *et al.*, 2003). The volume of water used for toilet flushing was assumed to be 34L per person per day (Karpiscak *et al.*, 1990; Almeida *et al.*, 1999). With an average 2.4 persons per household, based on figures for the UK (Evans and Hartwich, 2005), 82L.d^{-1} and 49640L.year^{-1} water is used for toilet flushing by the average household. At an EO concentration of 500 µL.L^{-1}, a typical household of four would require 41mL EO per day and 15L of EO per year. Therefore, to provide sufficient EO to disinfect grey water in a typical household in the UK for the purpose of toilet flushing over a one year period, 3000m^2 of cultivated *Thymus capitatus* plants would need to be harvested. This compares to the average household size in the UK of 85m^2 (Evans and Hartwich, 2005), meaning that 35 times the average household land area would be required to produce the EO.

In terms of both the land area required and the anticipated costs, the disinfection of grey water with essential oils appears impractical. Treatment of the grey water to a higher standard, reducing the level of organics and particulate matter, is likely to reduce the EO concentration required for effective disinfection, thereby increasing the feasibility of using EOs as disinfectants, or regrowth inhibitors, for urban water reuse.

6.4. CONCLUSIONS

The data presented demonstrate a number of key points regarding the use of essential oils for the disinfection of grey water: (1) initial concentration and contact time are important determinants of the degree of coliform inactivation by origanum EO, (2) the efficacy of disinfection by origanum EO is limited by organic and particulate material in the water, and (3) origanum EO can prevent coliform regrowth in treated grey water reed bed effluent for up to 14 days at a concentrations of 468 mg.L^{-1}.

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CHAPTER 7:

OVERALL ISSUES FOR REUSE

7. OVERALL ISSUES FOR REUSE

The present thesis set out to understand the pathogen content of grey water and to evaluate pathogen removal by disinfection processes to provide water that is suitable for reuse. The preceding literature review and technical papers have all contributed towards this aim and include discussion on the implications for grey water reuse. Here, their findings are brought together for evaluation and further analysis of the implications for grey water reuse.

7.1. GREY WATER QUALITY

7.1.1. Pathogens in grey water

A review of the literature and experimental analysis on grey water provided insight into the pathogens present in grey water streams. Grey water consistently contains bacteria of faecal origin, regardless of the grey water stream, demonstrating the potential for a range of faecally-transmitted enteric bacterial, protozoan, and viral pathogens to be passed into grey water. This has been confirmed by studies reporting the presence of faecally transmitted protozoa and bacteria in grey water (Birks *et al.*, 2004; Birks and Hills, 2007). Although pathogenic viruses have not been reported in grey water, their presence can be anticipated.

The frequency with which these enteric pathogens are reported and the numbers detected suggest that enteric pathogens are typically present in grey water at low concentrations. This indicates that certain bacterial pathogens, such as *Salmonella* spp. may be of little concern for grey water reuse because of the relatively high number of bacterial cells, $>10^5$, required to cause infection (Kothary & Babu, 2001). However, the infective doses of enteric bacterial pathogens vary and some are considerably lower (Table 7.1.). For example, infective doses of *Campylobacter jejuni*, shiga toxin producing *Escherichia coli*, and *Shigella* spp., are 500-800, <100, and 10 cells, respectively (Black, *et al.*, 1988; Griffin and Tauxe, 1991; Crockett *et*

Table 7.1. *Potential pathogens in grey water and infective dose.*

Pathogen type	Pathogen	Major symptoms of disease	Transmission via water	Minimum infective dose (cells/particles)
Enteropathogenic bacteria	*Salmonella* spp.	Gastroenteritis, typhoid, paratyphoid	Ingestion	$>10^5$ (Kothary & Babu, 2001)
	Vibrio cholerae	Gastroenteritis	Ingestion	10^5 (Cohen *et al.*, 1999)
	Campylobacter jejuni	Gastroenteritis	Ingestion	500-800 (Black, *et al.*, 1988; Kothary & Babu, 2001)
	Shiga toxin-producing Escherichia coli (STEC)	Gastroenteritis, haemorrhagic colitis, haemolytic uraemic syndrome	Ingestion	<100 (Griffin and Tauxe, 1991)
	Shigella spp.	Gastroenteritis, dysentery	Ingestion	10 (Crockett *et al.*, 1996)
Opportunistic bacteria	*Pseudomonas aeruginosa*	Skin, eye and lung infections, gastroenteritis	Ingestion, inhalation, topical contact	ND
	Staphylococcus aureus	Boils, scalded skin syndrome, toxic shock syndrome, gastroenteritis, pneumonia	Ingestion, inhalation, topical contact	ND
	Legionella pneumophilia	Pneumonia	Inhalation	ND
	Mycobacterium spp.	Pulmonary disease	Inhalation	ND
	Clostridium perfringens	Gastroenteritis, gas gangrene.	Ingestion, topical contact	ND
Protozoa	*Cryptosporidium parvum*	Gastroenteritis	Ingestion	10 (Okhuysen *et al.*, 1999)
	Entamoeba spp.	Gastroenteritis, dysentery	Ingestion	1 (Kothary & Babu, 2001)
Viruses	Norovirus, Rotavirus	Gastroenteritis	Ingestion	1-10 (Leclerc *et al.*, 2004)

ND: no data

al., 1996; Kothary & Babu, 2001). Pathogenic protozoa and viruses also have low infective doses, with as little as 1 infectious unit capable of causing infection (Okhuysen *et al.*, 1999; Kothary & Babu, 2001; Leclerc *et al.*, 2004). The enteric pathogens with low infective doses are of most concern for grey water reuse as they hold the most potential to cause infection at the low concentrations likely to be found in grey water.

A number of opportunistic pathogens in grey water have been reported in the literature and in the present thesis. *Pseudomonas aeruginosa* and *Staphylococcus aureus* may be present in grey water in relatively high concentrations of up to 4.4 and 4.0 $\log_{10}.100mL^{-1}$, respectively (Casanova *et al.*, 2001; Gilboa and Friedler, 2007). The potential for infection to occur by these opportunistic pathogens is difficult to quantify but their presence in such high concentrations indicates a particular risk of grey water reuse to the vulnerable members of society who are more susceptible to infection by such pathogens.

7.1.2. Chemical and physical quality of grey water

The chemical and physical quality of grey water will also affect its suitability for reuse. Grey water contains a range of metals and organic chemicals, many of which are xenobiotic and may be toxic to plants or to humans (Eriksson *et al.*, 2003; Palmquist and Hanaeus, 2005). The toxic effects of these chemicals are typically cumulative and since reused grey water is not intended for human ingestion, the risk to users of reused grey water is likely to be low. Irrigation with untreated grey water can lead to an accumulation of surfactants in soil, creating water-repellent soils and negatively impacting flow patterns and plant productivity (Wiel-Shafran *et al.*, 2006). Salts and Boron in grey water are also a concern for irrigation as they can have a toxic effect on plants (Gross *et al.*, 2005; Wiel-Shafran *et al.*, 2006). The removal of surfactants and other contaminants from grey water should also be targeted by treatment processes to improve the application of grey water for reuse as irrigation water. The physical characteristics of grey water will also affect the aesthetic quality of the reused water. Turbidity, colour, and suspended solids in grey water will reduce its aesthetic appeal and negatively impact public acceptance of grey water reuse schemes.

7.2. GREY WATER DISINFECTION

7.2.1. Inactivation curves

To compare the disinfection of grey water and treated grey water with chlorine, UV light, and origanum essential oil, the applied disinfectant concentrations were converted to Mols.L^{-1}. Chlorine concentrations were converted to Mols.L^{-1} using the relative atomic mass of 71 for Cl_2. Ultraviolet (UV) light was converted to Mols of photons (mols of einstein) .L^{-1} by using:

$$E = hv$$

where: E = energy of a single photon in Joules; h = Planck's constant; and v = frequency.

The frequency was determined using the known wavelength of the UV light, 254nm, allowing calculation of the number of photons in 1 Joule of UV light and subsequently, the Mol of photons, using Avogadro's constant. Finally, knowing the surface area and volume of water exposed to UV light allowed calculation of the Mols of photons applied to the disinfected water. Origanum essential oil (EO) was converted to Mols.L^{-1} using the relative atomic mass of 150 for carvacrol and thymol, which are the key components for microbial inactivation (Lambert *et al.*, 2001; Peñalver *et al.*, 2005) and typically constitute between 54 and 83% of the reported components in the oil (Karpouhtsis *et al.*, 1998; Fleisher and Fleisher, 2002; Goren *et al.*, 2003).

Comparison of the disinfection of grey water by chlorine, UV light, and origanum EO, revealed that the molar concentrations causing inactivation of total coliforms in grey water were not drastically different for the three disinfectants (Figure 7.1.). Chlorine and UV light were both effective at low molar concentrations, causing >3 $\log_{10}$ inactivation of coliforms at 7.0×10^{-5} and 2.7×10^{-5} Mols.L^{-1}, respectively. In contrast, origanum EO was ineffective at low concentrations but provided >3 $\log_{10}$ inactivation of coliforms at 1.9×10^{-3} Mols.L^{-1}. The chlorine and UV light inactivation curves both

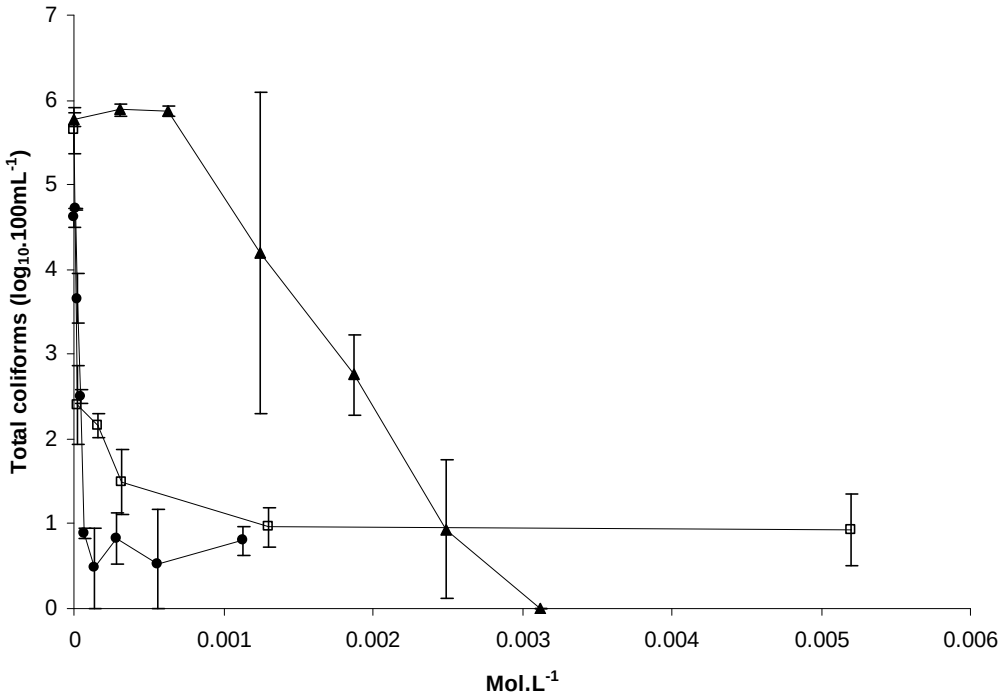

Figure 7.1. *Disinfection of grey water by chlorine (●), UV (□), and origanum EO (▲), expressed as Mols of disinfectant per litre. Contact time for chlorine and origanum EO was 30 minutes.*

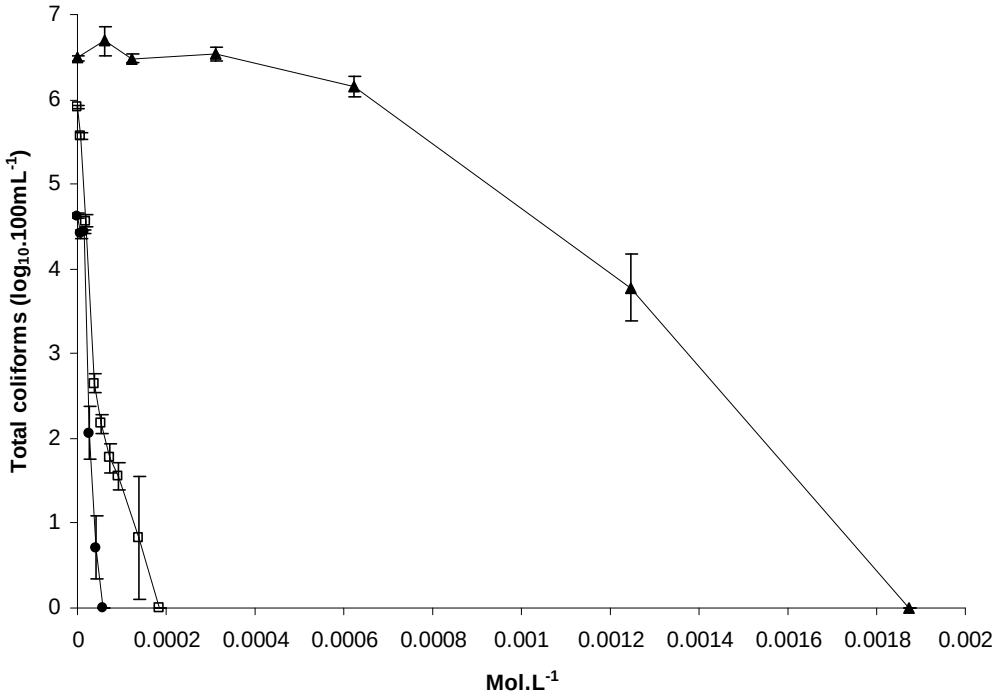

Figure 7.2. *Disinfection of VFRB- treated high strength grey water effluent by chlorine (●), UV (□), and origanum EO (▲), expressed as Mols of disinfectant per litre. Contact time for chlorine and origanum EO was 30 minutes.*

exhibited tailing, with a persisting coliform concentration of approx. 1.0 $\log_{10}.100mL^{-1}$ at concentrations of around 3.0×10^{-4} and greater. In contrast, coliforms were not detected in grey water with 3.1×10^{-3} $Mols.L^{-1}$ origanum EO. However, unlike chlorine and UV light, origanum EO could not be neutralised following the allocated disinfectant contact time. Despite efforts to minimise the effects of this (see Chapter 6, section 6.2.), it is possible that the origanum EO was able to continue to exert an antimicrobial influence during the incubation procedure to enumerate the coliform bacteria. Therefore, the data showing the complete inactivation of coliform bacteria by origanum EO should be received with caution.

Treatment of the supplemented 'high strength' grey water through a vertical flow reed bed (VFRB) produced treated effluent of superior quality compared to the low strength grey water. For instance, the VFRB effluent samples used for the disinfection experiments had COD and TSS values of up to 82 and 12 $mg.L^{-1}$, compared to 110 and 34 $mg.L^{-1}$ for the low strength grey water (Table 7.2.). However, the numbers of total coliforms in the treated effluent were similar to those of the low strength grey water samples used for disinfection experiments (Figures 7.1. and 7.2.). Chlorine and UV light provided >3 $\log_{10}$ inactivation of coliforms at 4.2×10^{-5} and 3.7×10^{-5} $Mols.L^{-1}$, respectively, similar to the concentrations required for >3 log inactivation in the untreated grey water (Figure 7.2.).

Table 7.2. *Water quality of grey water and VFRB-treated grey water. Data are ranges of values for water samples used in chlorine, UV, and origanum EO disinfection experiments.*

Water quality parameter	Grey water	VFRB-treated grey water
BOD ($mg.L^{-1}$)	19 – 20	ND
COD ($mg.L^{-1}$)	75 – 110	28 – 82
TSS ($mg.L^{-1}$)	19 – 34	<1 – 12
Turbidity (NTU)	18 – 40	2.1 – 2.2
Ammonia ($mg.L^{-1}$)	0.9 – 1.1	<0.5
pH	7.3 – 7.7	6.8 – 7.0
Mean particle size (µm)	148 – 505	36 – 120
UV transmittance (%, cm^{-1})	47	62

ND: no data

Complete inactivation of coliforms in the treated effluent was achieved by chlorine and UV light at 5.6×10^{-5} and 1.9×10^{-4} Mols.L^{-1}, respectively. The lack of significant tailing in the inactivation curves can be attributed to the low suspended solids in the treated effluents and the low mean particle size compared to the untreated grey water. The origanum EO inactivation curve shows a considerable lag phase, and a significant reduction in coliform number was only observed at a concentration of 1.2×10^{-3} Mols.L^{-1}. A concentration of 1.9×10^{-3} Mols.L^{-1} provided a >3 log inactivation, as with the untreated grey water, and also achieved complete inactivation of coliforms in the treated effluent.

7.2.2. Sensitivity to water quality

Comparison of the sensitivity of the disinfection technologies to particulate material in grey water revealed that all were affected by the particle size distribution of the grey water. Increasing mean particle size reduced the efficacy of disinfection (Figure 7.3.) by shielding coliforms from the applied disinfectant. Chlorine and UV disinfection is known to be negatively effected by the presence of organic material in water (Asano *et al.*, 2007). However, when the disinfectant dose was adjusted to account for the chlorine demand or reduced UV transmittance, no significant effect of additional organic material in grey water on the efficacy of disinfection was observed (Figure 7.4.). In the case of origanum EO, a consistent initial concentration was applied to grey water samples of increasing organic strength. A close relationship of increasing coliform survival with increasing organic concentration was observed. The data indicates that organic material interacts with either origanum EO or coliform bacteria, reducing the potency of disinfection. By quantifying this interaction, the applied dose of origanum EO could be adjusted in a similar manner to chlorine and UV to account for the negative effect of organic material on disinfection.

It has been demonstrated that chemical and physical pollutants in grey water can impact the efficacy of disinfection processes, particularly organic and particulate material. Other important characteristics of grey water that can impact disinfection are pH, ammonia, and certain metal ions.

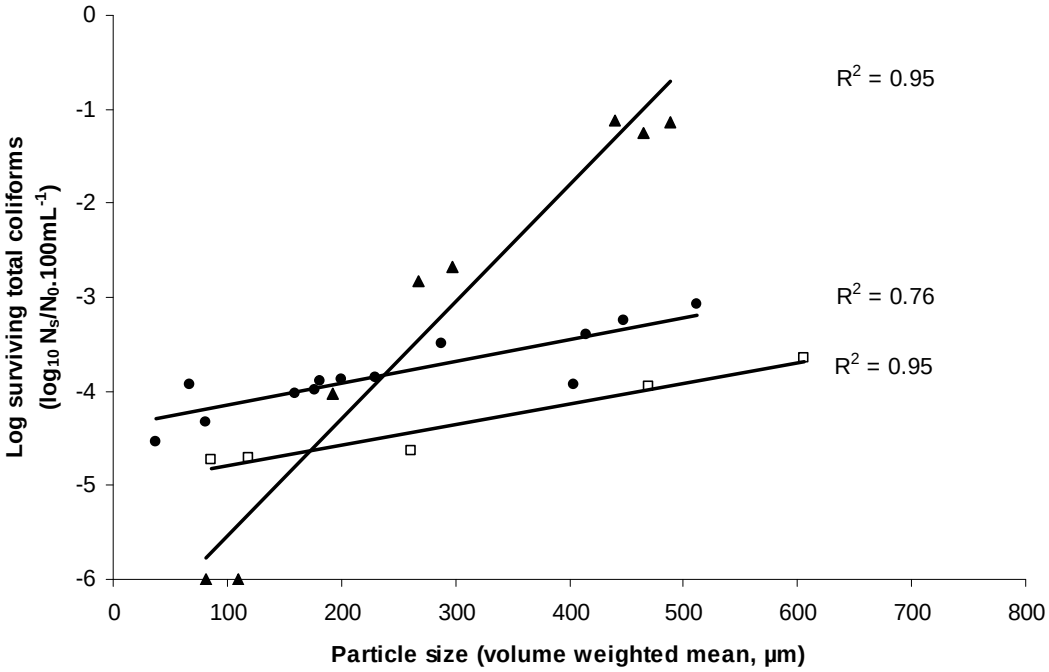

Figure 7.3. *Effect of particle size in grey water on disinfection by chlorine (●), UV (□), and origanum EO (▲). Doses applied: Chlorine 600 mg.min.L^{-1} (initial chlorine), UV 364 mJ.cm^{-2}, EO 8430 mg.min.L^{-1}. Contact time for chlorine and origanum EO was 30 minutes.*

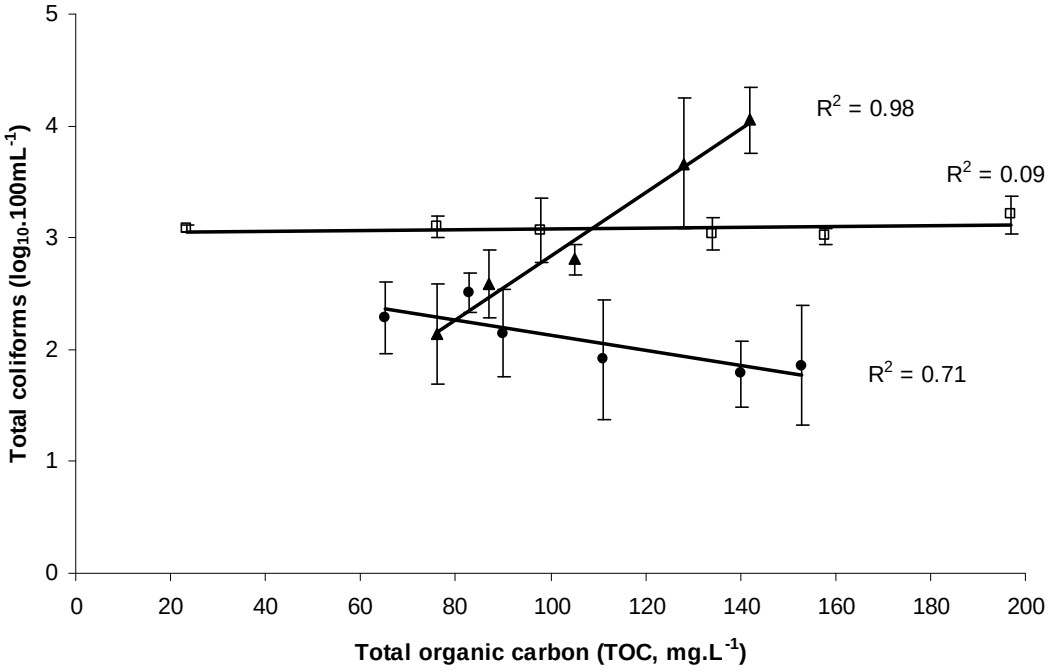

Figure 7.4. *Effect of organics in grey water on disinfection by chlorine (●), UV (□), and origanum EO (▲).Doses applied: Chlorine 30 mg.min.L^{-1} (free chlorine residual), UV 21 mJ.cm^{-2}, EO 8430 mg.min.L^{-1}. Contact time for chlorine and origanum EO was 30 minutes.*

The pH of grey water is typically between 6 and 8, however, it can vary, from as low as 5.2 and up to 10.0 (Chapter 2, Table 2.1.). Disinfection with chlorine is strongly affected by pH, with an increase in pH above 7 reducing the efficacy of disinfection. pH control of grey water may therefore be necessary to ensure effective disinfection with chlorine. Ammonia concentrations in grey water are also highly variable, from <0.1 to 15.0 $mg.L^{-1}$ (Chapter 2, Table 2.1.). Free chlorine applied to grey water will react with ammonia to form chloramines, which have reduced disinfecting power compared to free chlorine. Longer chlorine contact times may be necessary to achieve an equivalent level of disinfection in grey water when ammonia is present in sufficient concentrations. Disinfection by UV light is largely unaffected by the pH and ammonia values seen in grey water and this is an advantage for this method of disinfection over chlorine. Metal ions, such as Fe and Mn, will also react with free chlorine, reducing the concentration available for disinfection. The disinfection of grey water by UV light is also limited in the presence of Fe as it is a strongly UV-absorbing substance, which can reduce UV transmittance through the water.

7.2.3. Disinfection kinetics

Total coliform inactivation rate constants were determined using Chick's (1908) law:

$$\frac{dN_t}{dt} = kN_t$$

where: dN_t/dt = the rate of change in the number of coliforms with time; N_t = the number of coliforms at time t; k = inactivation rate constant; and t = time.

Disinfectant dose, as $mg.min.L^{-1}$ for chlorine and origanum EO, and $mJ.cm^{-2}$ for UV, was plotted against $\log_{10} (N_s/N_0)$, where N_s = the number of surviving coliforms, and N_0 = the initial number of coliforms. The inactivation rate constant (k) was determined by the gradient of the line of best fit for both the linear and tailing regions of the inactivation curves.

In the untreated grey water, the linear phase inactivation rate was highest for UV, at 0.27 $cm^2.mJ^{-1}$, followed by chlorine at 0.03 $L.mg^{-1}.min^{-1}$, and origanum EO at less than 0.001 $L.mg^{-1}.min^{-1}$ (Table 7.3.) The tailing phase inactivation rates for chlorine and UV were very low, at just 0.0002. In the VFRB-treated grey water, the linear phase inactivation rate constant was increased for chlorine, to 0.06 $L.mg^{-1}.min^{-1}$, and slightly reduced for UV to 0.20 $cm^2.mJ^{-1}$., compared to the untreated grey water (Table 7.3.). The greatest difference was observed in the tailing phases, however, where inactivation rate constants were 0.0164 and 0.0315 for chlorine and UV, respectively, compared to 0.0002 for the untreated grey water. The data demonstrates that, following the linear phase in the treated grey water, coliforms continued to be inactivated, albeit at a slower rate, unlike in the untreated grey water where coliforms persisted at high doses (Figure 7.1.). The reduced impact of the tailing region on disinfection of the treated effluent can be attributed to the reduction in suspended solids concentration and particle size by the treatment process (Table 7.2.).

Table 7.3. *Inactivation rate constants (k) for total coliform bacteria in grey water and treated grey water.*

Disinfectant	Grey water		VFRB-treated grey water effluent	
	Linear phase	Tailing phase	Linear phase	Tailing phase
Chlorine ($L.mg^{-1}.min^{-1}$)	0.0315	0.0002	0.0608	0.0164
UV light ($cm^2.mJ^{-1}$)	0.2657	0.0002	0.2035	0.0315
Origanum EO ($L.mg^{-1}.min^{-1}$)	0.0005	n/a	0.0010	n/a

7.3. APPLICATION FOR REUSE

7.3.1. Disinfection to reuse standards

Regional microbiological standards for urban water reuse vary considerably but they usually include indicator bacteria, typically coliforms, as determinants of microbiological quality. The disinfectant dosages required to achieve defined total

coliform disinfection standards vary accordingly. For example, to achieve a 1000.100mL^{-1} total coliform standard in secondary activated sludge wastewater effluent, a UV dose of 40-50 mJ.cm^{-2} is needed, but to achieve ≤2.2 coliforms .100mL^{-1}, the required dose increases to 90-110 mJ.cm^{-2} (Asano *et al.*, 2007; Table 7.4.).

The chlorine and UV doses observed to give total coliform levels of 200 or 1000 .100ml^{-1} in grey water were generally lower than those of wastewater effluent. For instance, 113 mg.min.L^{-1} and 43 mJ.cm^{-2} chlorine and UV doses, respectively, gave 200 coliforms .100mL^{-1}, compared to around 150-450 mg.min.L^{-1} and 50-70 mJ.cm^{-2} for wastewater effluent (Table 7.4.). Neither chlorine nor UV was able to reach the ≤2.2 coliforms .100mL^{-1} standard in grey water at the maximum doses applied of 2400mg.min.L^{-1} and 2341 mJ.cm^{-2}, respectively. The disinfection of treated grey water had little effect on the chlorine and UV doses required to reach the 1000 coliforms .100mL^{-1} standard but greatly reduced the doses needed to achieve the more stringent criteria of 23 and ≤2.2 coliforms .100mL^{-1}.

Table 7.4. *Disinfectant doses required to achieve different total coliform disinfection standards in untreated and treated grey water, compared against secondary wastewater effluent.*

Disinfectant	**Water type**	**Initial dosage to achieve total coliform (.100mL^{-1}) levels:**			
		1000	200	23	≤2.2
Chlorine (mg.min.L^{-1})	WW	60 – 300	150 – 450	300 – 900	ND
	GW	77	113	147	ND
	TGW	59	60	86	118
UV light (mJ.cm^{-2})	WW	40 – 50	50 – 70	70 – 90	90 – 110
	GW	12.3	43	334	ND
	TGW	16.5	23.5	52	77
Origanum EO (mg.min.L^{-1})	WW	ND	ND	ND	ND
	GW	8348	10290	11150	13410
	TGW	7950	8330	8380	8420

WW: Secondary activated sludge wastewater effluent (10^{5}-10^{6} initial total coliform concentration .100mL^{-1}), adapted from Asano et al. (2007).
GW: Grey water (10^{4}-10^{6} initial total coliform concentration .100mL^{-1})
TGW: Treated grey water, VFRB effluent (10^{4}-10^{6} initial total coliform concentration .100mL^{-1})
ND: no data

For instance, the ≤2.2 coliforms .100mL^{-1} standard was reached in treated grey water at chlorine and UV doses of 118 mg.min.L^{-1} and 77 mJ.cm^{-2}, respectively. Importantly, this data demonstrates that the disinfection of grey water by chlorine or UV light to meet stringent standards for urban reuse is not achievable without a prior treatment stage.

The origanum EO doses were very high, at 8000 mg.min.L^{-1} or more to meet the 1000 coliforms .100mL^{-1} standard in untreated or treated grey water. The treated grey water reduced the doses required; suggesting that origanum EO may be a more appropriate disinfectant for higher quality treated effluents, such as MBR-treated grey water, for example. Further investigation is required to explore this possibility.

7.3.2. Cost analysis of grey water disinfection

A comparison of the potential costs for disinfecting grey water was made. For small-scale grey water reuse, such as on an individual home basis, the high capital costs of UV disinfection are likely to be a deterrent, whereas, chemical disinfectants can be applied manually, avoiding the expense of a dosing system. While UV disinfection is unlikely to be cost competitive with chlorine disinfection for small-scale water reuse, at a larger scale the cost difference becomes smaller, although chlorine remains the most economical (Hendricks, 2006). A comparison of the costs of using chlorine or origanum EO was made, for the disinfection of both untreated and treated grey water for the purpose of toilet flushing.

The volume of water used for toilet flushing was assumed to be 34L per person per day (Karpiscak *et al.*, 1990; Almeida *et al.*, 1999) and a disinfectant contact time of 30 minutes was assumed. The disinfectant doses required to meet total coliform standards of 1000 and 23 .100mL^{-1} were selected for comparison (Table 7.4.). Chlorine costs were based on a calcium hypochlorite cost of £95 for 20kg (Swimmingpoolchemicals.co.uk, 2007) and an assumption of 70% free chlorine (Tchobanoglous *et al.*, 2003), giving a cost of £6.79 per kg free chlorine. Origanum oil costs were based on a cost £400 for 5kg

(Sigma-Aldrich, 2007), giving £80 per kg. UV light costs were based on an electricity cost of £0.085 per kW.h (DTI. 2006).

Table 7.5. *Comparison of potential chlorine, origanum essential oil, and UV light costs for the disinfection of untreated, and treated, grey water.*

Water source	Total coliform standard (.100mL^{-1})	Chlorine		Origanum EO		UV light	
		Cost (£) per m^3	Cost (£) per person per year	Cost (£) per m^3	Cost (£) per person per year	Cost (£) per m^3	Cost (£) per person per year
Grey water	1000	0.018	0.216	22.24	276.26	0.0029	0.036
	23	0.033	0.413	29.76	368.99	0.0789	0.979
Treated grey water	1000	0.013	0.166	21.20	263.09	0.0038	0.047
	23	0.019	0.242	22.32	277.32	0.0123	0.153

The calculated disinfectant costs of chlorine and origanum EO were very different, with disinfection of grey water by chlorine costing up to £0.03.m^{-3} compared to £29.76.m^{-3} for origanum EO, around three orders of magnitude greater (Table 7.5.). UV light disinfection costs were generally lower than those of chlorine, with the exception of disinfecting grey water to the 23 total coliform .100ml^{-1} standard, which was almost double the cost of chlorine disinfection. The lower disinfectant concentrations required to provide equivalent inactivation of total coliforms in treated grey water effluent leads to lower costs for disinfection. Also, the % increase in cost required to disinfect to the 23 total coliform standard from the 1000 total coliform standard was 83% in grey water, compared to just 46% in treated grey water. Combining the cost of £0.034.m^{-3} for the VFRB with the cost of chlorine disinfection of £0.027.m^{-3} to achieve $\leq$2.2 total coliforms per 100mL gives a total operating cost of £0.061.m^{-3}, equivalent to just £0.76 per person per year.

While these costs allow comparison of the relative likely expense of using chlorine or origanum EO for disinfection, they only represent operating costs and capital costs are not considered. The costs calculated assume that the exact quantity of water used for toilet flushing is disinfected with the exact dose required to achieve a level of coliforms determined from laboratory experiments. The disinfectant quantities required are

therefore likely to have been underestimated compared to real systems. Also, the potential disinfectant decay over contact times of greater than 30 minutes is not accounted for. The actual costs involved can therefore be expected to be greater in real-world application of these disinfectants for urban water reuse.

7.3.3. Other considerations

Other considerations for grey water disinfection and reuse include the maintenance of a disinfectant residual, the toxicity of the disinfected grey water, and public acceptance of grey water reuse schemes. Disinfection by UV light provides no residual disinfection, allowing the possibility of regrowth of bacteria. Chlorine has also been shown to decay in grey water with increasing storage time. The solution is to treat the grey water to reduce the levels of organics and the potential for bacterial regrowth, or to minimise storage times of disinfected grey water prior to reuse, reducing the opportunity for regrowth. The addition of chlorine or essential oil to grey water increases its potential toxicity to humans and the environment. Chlorine will react with organics in the grey water to form disinfection by-products, which pose a risk to human health (refs) and essential oils contain components which may be toxic to the environment, as indicated in chapter 6 of the present thesis. Disinfection by UV light holds an advantage over the chemical disinfectants because it is not known to cause the production of toxic compounds.

7.3.4. Public acceptance of grey water reuse and disinfection

The intended application for reused water influences public acceptability. For example, the use of reclaimed water for drinking water or for food preparation receives most opposition, while use for irrigation of recreational parks and golf courses attracts the least public resistance (Asano *et al.*, 2007). The principal applications of grey water reuse, toilet flushing and garden irrigation, appear not to be of particular concern to the public. For instance, surveys of public opinion on the uses of reclaimed water indicate that typically, less than 4% of individuals are opposed to use for toilet flushing or

garden irrigation, compared to up to 30% who are opposed to use for home laundry (Asano *et al.*, 2007).

Public perception of the relative environmental credentials of disinfection technologies for grey water reuse may also impact technology selection. For instance, in Germany, chemical disinfection is seen as unfavourable for urban water reuse and so UV disinfection systems are preferred for the disinfection of grey water (Nolde, 2005). There is no simple method for determining the 'environmental friendliness' of the disinfection technologies considered in the present thesis. Chlorine disinfection can result in the formation of harmful disinfection by-products and plant essential oils frequently contain high concentrations of phenols and other chemicals which are considered toxic to the environment. While disinfection with chlorine and essential oils involves the use of chemicals and their eventual discharge to the environment, ultraviolet light requires the use of significant amounts of energy, which may negatively impact the environment through the process of energy production. UV lamps also contain small quantities of mercury, an element which is considered hazardous to the environment. Evaluating the relative impacts of disinfection technologies on the environment is therefore not straight-forward. Public perception and acceptance is, however, likely to be a strong factor in determining the feasibility of water reuse schemes. The real and perceived environmental impacts of disinfection technologies for grey water must also be balanced against costs and the need for effective disinfection.

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CHAPTER 8:
CONCLUSIONS AND FURTHER WORK

8. CONCLUSIONS AND FURTHER WORK

8.1. CONCLUSIONS

The present thesis has extended understanding of the microbial quality of grey water, including the potential presence of different types of pathogenic microorganisms. A greater understanding of the impact of grey water quality, specifically organic and particulate material, on leading contender treatment and disinfection technologies, has also been established. The following conclusions can be drawn from this work:

1. A review of the literature and experimental analysis revealed that grey water is consistently contaminated with faecal material and can contain enteric pathogens of concern, including *Salmonella* spp., and *Cryptosporidium*, although in low concentrations. Opportunistic pathogens, infectious by inhalation or topical contact, such as *Pseudomonas aeruginosa* and *Staphylococcus aureus*, are typically present in grey water in significant numbers and indicate a particular risk of grey water reuse to the elderly, very young, and immunocompromised members of society. (Objectives 1 and 2)

2. The aerobic, unsaturated flow conditions of the vertical flow reed bed (VFRB) provided the best and most robust microorganism and pollutant removal from grey water, of the three configurations of constructed wetland tested. Overall, the membrane bioreactor (MBR) was the most robust treatment and disinfection technology. (Objective 3)

3. Total coliform bacteria were good indicators of *P. aeruginosa* in grey water and for its removal through treatment processes. (Objective 3)

4. Grey water contains large particles (up to 2000µm) with particle-associated microorganisms, which are resistant to disinfection. The efficacy of chlorine and ultraviolet (UV) light disinfection was closely linked to the particle size

distribution in grey water and, for UV; the proportion of coliform bacteria shielded from inactivation was greater at a larger mean particle size. (Objectives 2, 4 and 5)

5. The presence of additional organic material in grey water increased chlorine demand and reduced UV transmittance but did not increase the resistance of coliform bacteria to disinfection when a constant chlorine or UV dose was applied. (Objectives 4 and 5)

6. The efficacy of origanum essential oil was strongly inhibited by the presence of organic material. Essential oils can provide effective disinfection of grey water but high doses are required in comparison to chlorine and they are therefore likely to be financially unfeasible for the disinfection of grey water for reuse. (Objectives 4 and 5)

7. In order to meet stringent microbiological requirements for urban reuse, grey water should first be treated to remove the larger particulate material that limits the disinfection process. The removal of organic material will also reduce the doses required for an equivalent level of disinfection. (Objective 5)

8.2. FURTHER WORK

A number of areas where further research would be beneficial have been identified during the course of this research. These are detailed below:

1. Large volume testing for specific pathogens from numerous grey water sources would provide more detailed information on the frequency and concentrations of pathogens in grey water, allowing for accurate risk assessment and informing requirements for treatment and disinfection technologies.

2. Assessing the potential for pathogen transmission from urban water reuse applications, such as via aerosols from toilet flushing, would further inform risk assessment and disinfection requirements for grey water reuse.

3. Analysis of the inactivation of specific pathogens, including protozoa and viruses, in grey water by disinfection processes would be beneficial in determining whether disinfection to coliform standards will produce the pathogen-free water desired for reuse.

4. This research identified particle-associated coliform bacteria as being resistant to inactivation and limiting disinfection. Further study of the extent to which specific pathogens become particle-associated and are shielded from disinfection would be of interest.

5. Organic material was shown to impact the efficacy of disinfection by essential oils. It is not known whether this was the result of a 'demand' effect, in which the essential oil was rendered inactive by the organic material or whether the organic material affected the targeted microorganisms, increasing their resistance to inactivation by the essential oil. Further study would illuminate the mechanisms of microbial inactivation of essential oils.

6. Origanum essential oil was shown to require high doses to provide inactivation of coliforms in grey water and treated effluent. Both of these water sources still contained significant levels of organic material. The efficacy of origanum essential oil for disinfection or inhibition of regrowth in a higher quality treated effluent, an MBR effluent for example, should be investigated before essential oils are considered unsuitable disinfectants for water reuse.